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PROCEEDINGS

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SERIES B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

VOL. LXXXV.

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THE ROYAL SOCIETY.

Section B.—Biological Sciences.

An Antelope Trypanosome.

By Captain A. D. Fraser, R.A.M.C., and Dr. H. L. Duke.

(Communicated by Sir J. Rose Bradford, Sec. R.S., being an abstract of a Report to the Sleeping Sickness Commission, received May 19, 1911. Received December 19, 1911,—Read January 18, 1912.)

Ten days after blood of a bushbuck, which was shot on the shores of the Victoria Nyanza, had been injected into a healthy goat, trypanosomes appeared in the goat's blood. The same species of trypanosome was present in blood smears made from another bushbuck and a situtunga, which were shot in the same neighbourhood.

The small characteristic trypanosome corresponds morphologically to the one which was discovered in cattle in Uganda, and which was named *Trypanosoma uniforme* by the Royal Society Sleeping Sickness Commission, 1908–10. This is shown by curves representing the distribution, by percentages, in respect to length of the antelope trypanosome and *Trypanosoma uniforme.*

Cattle, goats, sheep, and bushbuck were infected. Monkeys, pigs, dogs, cats, guinea-pigs, and white rats proved to be refractory. It is concluded that the trypanosome found in the antelope was *Trypanosoma uniforme.*

Experimentally it was shown that laboratory-bred *Glossina palpalis* were capable of transmitting this species of trypanosome from infected to healthy animals. Of six experiments four were successful. The flies became infected in from 27 to 37 days, and the infection in the fly was always limited to the proboscis.

In order to ascertain if *Glossina palpalis* caught on the Lake-shore, near where the infected antelope had been shot, were naturally infected, flies were collected there and brought to Mpumu, where they were fed on a healthy goat. After 1020 flies had been put on the goat it became infected with *Trypanosoma uniforme.* Some days afterwards *Trypanosoma vivax,* with which wild flies had previously been shown to be naturally infected, also appeared in the goat's blood.

The conclusions are:—

(1) This trypanosome, which is of fairly frequent occurrence among Lake-shore antelope, is *Trypanosoma uniforme.*

(2) The available evidence points to *Glossina palpalis* as being the carrier of this species of trypanosome.

(3) *Glossina palpalis* caught on the Lake-shore are naturally infected with *Trypanosoma uniforme.*

The Relation of Wild Animals to Trypanosomiasis.

By Captain A. D. Fraser, R.A.M.C., and Dr. H. L. Duke.

(Communicated by Sir J. R. Bradford, Sec. R.S., being an abstract of a Report to the Sleeping Sickness Commission, received May 19, 1911. Received December 19, 1911,—Read February 29, 1912.)

Wild animals were examined with the view of ascertaining whether they were naturally infected with trypanosomiasis. The majority of the animals were shot; a few were captured.

Ten waterbuck, 20 bushbuck, and 2 situtunga were obtained from within two miles of the Lake-shore where the *Glossina palpalis* were known to be infected with *Trypanosoma gambiense* and *vivax* and were afterwards shown to be infected with *T. uniforme.* With the exception of four of the bushbuck ordinary blood smears were made from each animal, and trypanosomes morphologically indistinguishable from *T. uniforme* were found in slides taken from one bushbuck and one situtunga. Blood from each of the antelope was injected into healthy monkeys and goats. From 3 to 5 c.c. was the usual quantity of blood injected into monkeys; 5–10 c.c. into goats. None of the monkeys developed trypanosomiasis. In the blood of one of the goats *T. uniforme* appeared 10 days after the injection of blood of two bushbuck shot near the Lake. Unfortunately no blood smears were made from these animals. It is suggested that, if animals more susceptible than goats are to *T. uniforme* had been employed, a higher percentage of the antelope examined might have been shown to harbour this species of trypanosome.

Two bushbuck and one situtunga were also examined by feeding clean laboratory-bred *G. palpalis* upon them and subsequently transferring the flies to normal monkeys. The monkeys remained healthy and no flagellates were found in the flies which were dissected.

Twenty-two hippopotami, 8 crocodiles, 12 monitors, 60 Lake-shore birds, 2 bushpigs, 6 otters, etc., were examined, both microscopically and by blood injection into susceptible animals, with negative results.

G. palpalis were seen to feed on bushbuck and monitors in nature.

The animals obtained from a distance greater than two miles from the Lake-shore included 5 bushbuck, 2 duiker, and 3 bushpigs. *T. ingens* was present in slides made from 1 bushbuck.

Experiments were also carried out to ascertain whether bushpigs, etc., could be infected with *T. gambiense.* Blood from monkeys heavily infected with this trypanosome was injected into two young bushpigs. Cages of *G. palpalis* known to be infected with *T. gambiense* were also fed upon the pigs. No evidence was obtained that the pigs became infected.

Negative results were also obtained of attempts to infect a young crocodile, a monitor, a frog, and fowls. An edible rat was infected by injection.

Conclusions.

1. *T. uniforme* was the only species of trypanosome obtained as the result of examination of wild animals including 32 Lake-shore antelope.

2. The available evidence points to bushpig, crocodile, monitor, frog and fowls being refractory to *T. gambiense.*

3. The edible rat, which is susceptible to *T. gambiense,* can, by virtue of its habits, be of little importance in considering the question of a reservoir.

The Transmission of Trypanosoma nanum (*Laveran*).

By Dr. H. L. Duke.

(Communicated by Sir J. Rose Bradford, Sec. R.S., being a Report to the Sleeping Sickness Commission. Received December 19, 1911,—Read February 29, 1912.)

On March 17, 1911, Dr. van Someren kindly sent a goat to Mpumu, which he had inoculated with a trypanosome found by him in cattle in the neighbourhood of Sebwe River.

This trypanosome, he averred, caused a fatal disease. On further investigation, it proved to be identical with *Trypanosoma nanum*, both as regards morphology and animal reactions, and thus confirms the opinion of the 1908–10 Commission, namely, that this trypanosome is a Uganda species.

Although experiments are still in progress, sufficiently important results have already been obtained to warrant their being recorded, adding, as they do, one more to the long list of trypanosomes carried by *Glossina palpalis*.

Morphology.

A. *Living, Unstained.*

Corresponds closely to *Trypanosoma pecorum*. The tendency to stick to adjacent corpuscles is very marked.

B. *Fixed and Stained* (*Osmic and Giemsa*).

As regards general structure, agrees with the description given by former workers in this laboratory. No trace of a free flagellum is visible.

Few observations are available as regards length, owing to the limited number of susceptible animals inoculated.

The following table gives maximum, minimum, and average measurements of the trypanosome in ox and sheep :—

Experiment No.	Animal.	Fixation.	Stain.	In microns.		
				Maximum.	Minimum.	Average.
59	Sheep	Osmic and abs. alcohol.	Giemsa	16	12·00	14·5
232	Ox	”	”	15·5	11·00	13·5

ANIMAL REACTIONS.

The original goat (688) sent by Dr. van Someren shortly after inoculation is at present (October 20, 1911) apparently in excellent health. Trypanosomes are visible in its blood only at rare intervals.

The following animals were sub-inoculated from goat, Experiment 688 :—

Date.	Animal.	Incubation, in days.	Duration of disease.	Remarks.
17.3.11......	Monkey 689	—	—	No trypanosomes seen for 54 days.
27.3.11......	„ 712	—	—	„ „ 54 „
15.4.11......	„ 7	—	—	„ „ 38 „
17.3.11......	White rat 690	—	—	„ „ 63 „
27.3.11......	„ 713	—	—	„ „ 50 „
19.4.11......	„ 12	—	—	„ „ 34 „
19.4.11......	Puppy 11	—	—	„ „ 49 „
26.5.11......	„ 89	—	—	„ „ 60 „
17.5.11......	Wild pig	—	—	„ „ 60 „
16.5.11......	Sheep 59	10	—	In good health after 154 days.
17.5.11......	Goat 64	6	64	Died of *T. nanum.*

Transmission of Trypanosoma nanum *by* Glossina palpalis.

In the following experiments, laboratory-bred flies were employed, hatched from pupæ brought from Damba Island. The first six attempts at transmission failed, and the brief summary of these experiments given in Table I will suffice.

It will be noticed that in every case the flies were first fed upon Goat 688, the animal received from Toro.

Table I.

Experiment No.	Date of commencement.	Number of days.		Number of flies.		Result.	Animal on which flies were fed.
		On Goat 688.	Experiment lasted.	At 30th day.	Dissected during experiment.		
25	27.4.11	10	50	16	17	—	Normal Calf 43
27	29.4.11	8	48	19	22	—	„
32	1.5.11	6	49	12	16	—	„
36	3.5.11	4	47	28	28	—	„
57	15.5.11	3	56	35	47	—	„
86	25.5.11	7	47	30	43	—	„

A further set of experiments were now started and Sheep 59 employed for the first time for the infecting feeds. It was found that the flies fed much more readily upon the sheep, and, as will be seen below, a considerable number of positive flies resulted.

A brief recapitulation of each experiment is given below, the description of the positive flies being dealt with in Table II :—

Experiment 213.

Date.	Day of expt.	Procedure.	Result.	Remarks.
1911. July 21—29	1—8	Fed on Sheep 59............	...	*T. nanum* ++ in Sheep 59 on July 19, 21, 22, 25
July 30	9	Starved.		
July 31—Aug. 16 ...	10—26	Fed on normal Calf 232 ...	—	
Aug. 17	27	Starved.		
Aug. 18—Sept. 5 ...	28—46	Fed on Goat 307	—	54 flies alive on 30th day of experiment.
Sept. 6..................	47	Starved and dissected	...	Remaining flies, ♂ 10 ♀ 14.

Note.—Of the 62 flies dissected during this experiment one female was found infected (*cf.* Table II).

Experiment 235.

Date.	Day of expt.	Procedure.	Result.	Remarks.
1911. July 31—Aug. 3 ...	1—3	Fed on Sheep 59	...	*T. nanum* ++, in Sheep 59 on Aug. 2 and 3.
Aug. 4..................	4	Starved.		
Aug. 5—Sept. 16 ...	5—47	Fed on normal Calf 232...	+ (?)	62 flies alive on 30th day of experiment.
Sept. 17	48	Starved .		
Sept. 18—21	49—52	Fed on Dog X	...	Dog X died 22.9.11.
Sept. 22—26	53—57	Fed on Dog 399	—	Dog negative to trypanosomes for 27 days.
Sept. 27	58	Starved and dissected......	...	Remaining flies, ♂ 2 ♀ 27.

Note.—83 flies were dissected in the course of this experiment and of these 4 females were found to contain flagellates on the 58th day of the experiment (*cf.* Table II).

Experiment 238.

Date.	Day of expt.	Procedure.	Result.	Remarks.
1911. Aug. 2—3	1	Fed on Goat 688	...	*T. nanum* + in Goat 688 on Aug. 2.
Aug. 4..................	2	Starved.		
Aug. 5—Sept. 16 ...	3—45	Fed on normal Calf 232...	+ (?)	61 flies alive on 30th day. Calf showed *T. nanum* 18.9.11.
Sept. 17	46	Starved.		
Sept. 18—21	47—50	Fed on Dog X...............	...	Dog X died 22.9.11.
Sept. 22—26	51—55	Fed on Dog 399	—	Dog negative to trypanosomes for 27 days.
Sept. 27	56	Starved.		
Sept. 28—Oct. 3 ...	57—64	Fed on Bushbuck 396 ...	—	Negative to trypanosomes for 20 days.
Oct. 4—5	65—66	Starved and dissected......	...	Remaining flies, ♂ 16 ♀ 14.

Note.—65 flies dissected during this experiment. One female found positive (*cf.* Table II).

Experiment 243.

Date.	Day of expt.	Procedure.	Result.	Remarks.
1911. Aug. 3—5	1—2	Fed on Sheep 59	...	*T. nanum* + + in sheep on Aug. 3 and 5.
Aug. 6..................	3	Starved.		
Aug. 7—Sept. 16 ...	4—44	Fed on normal Calf 232...	+ (?)	77 flies alive on 30th day. Calf showed *T. nanum* on 18.9.11.
Sept. 17	45	Starved.		
Sept. 18—21	46—49	Fed on Dog X...............	...	Dog X died 22.9.11.
Sept. 22—26	50—54	Fed on Dog 399	—	Dog negative to trypanosomes for 27 days.
Sept. 27	55	Starved.		
Sept. 28—Oct. 3......	56—61	Fed on Bushbuck 396......	—	Bushbuck negative to trypanosomes for 20 days.
Oct. 4—7	62—65	Fed on cock.		
Oct. 8—9	66—67	Starved and dissected.	...	Remaining flies, ♂ 7, ♀ 13.

Note.—Total flies dissected in this experiment 112, of which 6 females and 3 males were found infected with flagellates (*cf.* Table II).

It will be seen that in the above four experiments the flies were fed upon Calf 232, which became infected about September 10 or 11. It is thus impossible to decide which experiment actually caused the infection, though some evidence on this point may be gathered from Table II.

Dissection of the Positive Flies of the above Experiments.

All positive flies were examined by Miss Robertson, and an account of the morphology of the flagellates will appear in due course in her report. I have here to express my obligation for information regarding the parts of the alimentary tract infected, with which Miss Robertson has supplied me throughout.

In the following Table II the flies are arranged in a progressive series, according to their age when dissected, all the flies used being newly hatched at the commencement of the experiment. The "+" and "–" signs are used to indicate presence or absence of flagellates in the various regions of the alimentary canal, and the intensity of an infection is indicated by repetition of the "+" sign. The symbol "0" denotes that no observations were recorded concerning that portion of the gut:—

Table II.

Date.	No. of fly.	Age of fly when dissected.	No. of experiment.	Sex.	Region of gut.					—
					Hind gut.	Thoracic gut to junction with proventriculus.	Proventriculus.	Salivary glands.	Proboscis.	Injection into Goat 329.
1.8.11	1	11th day	213	♀	+ +	–	–	–	–	
17.8.11	2	14th "	243	♀	+ +	+ + +	–	–	–	
25.8.11	3	22nd "	243	♀	+ + +	–	–	–	–	
28.8.11	4	25th "	243	♀	+ + +	+ + +	+ + +	–	+ + +	Contents of proventriculus injected into Goat 329.
29.8.11	5	26th "	243	♀	+ + +	+ +	+	–	–	
5.9.11	6	33rd "	243	♀	+ + +	+ + +	0	–	+ + +	
18.9.11	7	46th "	243	♂	+ + +	0	+	–	–	
20.9.11	8	48th "	243	♂	+ + +	0	–	–	+	
20.9.11	9	48th "	243	♀	+ +	–	–	–	–	
27.9.11	10	58th "	235	♀	+ + +	+ + +	+ + +	0	–	Proventriculus of each of these flies injected subcutaneously into Goat 329.
27.9.11	11	58th "	235	♀	+ + +	+ + +	+ + +	0	–	
27.9.11	12	58th "	235	♀	+ + +	0	–	0	–	
27.9.11	13	58th "	235	♀	+ + +	+ +	+ + +	0	+ +	
5.10.11	14	64th "	238	♀	+ + +	+ + +	+ +	–	+ + +	
9.10.11	15	67th "	243	♂	+ + +	–	–	–	–	

Note.—Goat 329, under examination 56 days, has never shown trypanosomes.

From the above it would appear that the development of *Trypanosoma nanum* in *Glossina palpalis* commences in the hind gut and extends forwards *viâ* the thoracic gut and proventriculus until finally the proboscis is reached. The salivary glands are apparently not invaded by this trypanosome. A fly with a negative proboscis is presumably not infective, this conclusion being supported by the fact that on three occasions injection of positive proventriculi failed to infect Goat 329.

Fly No. 4 shows that flagellates may be well established in the proboscis by the 25th day after the infecting feed, although apparently neither this fly nor No. 6 accounted for the infection of Calf 232 on September 10 to 11.

Fly No. 8 showed a few flagellates in the proboscis, while both proventriculus and thoracic gut were negative, and the proventricular infection of fly No. 14 was relatively slight in conjunction with a swarming proboscis. These suggest the possibility that infection of the proventriculus may be merely a temporary invasion, while the flagellates are becoming established in the proboscis, and not a permanent station, in which case the condition of Fly 8 is intelligible.

As regards the actual infecting fly in the above experiments the choice rests between Nos. 8, 13, and 14. All three flies were fed on Calf 232 from September 11 to 16, *i.e.* during the incubation period, and may thus have derived their flagellates secondarily from this source. This applies especially to Nos. 13 and 14. There is, however, no reason why both these flies should not have become infected originally at the commencement of the experiment, in which case differential diagnosis is impossible. In the case of flies Nos. 9 and 15 there can be little doubt that the flagellates were derived from Calf 232.

As regards the position of the flagellates in the proboscis, the labrum is the chief seat of infection, enormous numbers being found in this situation. In one instance only, fly No. 4, Table II, were trypanosomes observed in small numbers in the hypopharynx.

Conclusions.

(1) That the trypanosome received from Sebwe River* is *Trypanosoma nanum.*

(2) That this trypanosome can be transmitted by *Glossina palpalis,* the proportion of positive flies obtained being relatively large, and indicating that this fly may play an important part in the spread of the disease in Uganda.

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* Sebwe River is in Toro Province, Uganda Protectorate, in the neighbourhood of Lake George.

The Causes and Prevention of Miners' Nystagmus.

By T. Lister Llewellyn, M.D., B.S. (Lond.), Tyndall Research Student.

(Communicated by Dr. J. S. Haldane, F.R.S. Received December 19, 1911,—Read February 22, 1912.)

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Miners' nystagmus is an occupational neurosis which is confined to workers in coal mines. The chief symptom and physical sign is a rotatory oscillation of the eyeballs, which prevents the miner from accurately fixing anything towards which his vision is directed. The result may be compared to a cinematograph exhibition, in which the pictures have not been taken rapidly enough to produce a continuous image, or where the film has been worn away by over-use, giving a blurred image.

Description.

The man first notices that he is unable to perform the more skilled part of his work; he cannot notch timber well, and fails to drive his wedge, or strike with his pick the exact piece of coal aimed at. He next complains that the lamps dazzle his eyes, that he is unable to see anything at night time, and, finally, that the lamps and all surrounding objects are going round and round. Headache, varying from slight pain between the temples to attacks of extreme severity, giddiness on exertion and stooping, night-blindness, dread of light, and in severe cases marked nervous depression (two of my cases have expressed suicidal intentions), are all found in a marked case of nystagmus.

There are two distinct varieties of the disease, in the first of which the symptoms are absent or latent, and the man apparently suffers no disability. In the second group the man suffers more or less disability. Tables I and II, and a number of subsequent tables, are the result of an analysis of 400 consecutive cases which I have investigated up to the present time.

Table I.

Latent	43
Manifest	357

Table II.—Analysis of Symptoms.

Movements of objects	361		
Headache	305	Marked 38	Very marked in 16
Giddiness	308	„ 40	„ „ 7
Night-blindness	262	„ 4	
Photophobia	159	„ 9	

Physical Signs of the Disease.

Movements of Eyes.—These, in true miners' nystagmus, are of a rotatory nature, often irregular both in rhythm and extent, increased by exertion and by looking up, and diminished by looking downwards and inwards. The so-called lateral or vertical nystagmus is a rotatory one, with a large lateral or vertical excursion respectively. Both eyes are affected equally in the great majority of cases. I have seen well-marked movement in the stumps of very shrunken and useless eyes (Cases 15, 17). Rate, 80 to 500 a minute (Romiée (1), 120 to 500). Range of movement, 1 mm. to 1 cm. (Romiée (2)).

Movements of eyelids.—These are often marked, and, when present, render the detection of the nystagmus very difficult. This sign was present in 90 cases and marked in 17.

Tremor of the eyebrows, head, neck, and even of the shoulders, may also be present. Tremor of the head was present in 84 cases and marked in 22.

Stassen (3) suggests that the head tremor should be looked upon as antagonistic to the ocular movements, and designed to neutralise them. I think it should be regarded as a mark of extension of the disease. There is one very important sign to which sufficient attention has not been paid—the backward and often oblique position in which the head is held. This is accompanied with drooping of the upper eyelids and convergence of the eyes. I regard this sign as one of extreme importance.

The eyes are often congested, and a convergent squint is frequently found. The power of fixation and accommodation is greatly weakened. The patient tires very quickly, and the visual acuity may drop from normal to half of normal in a few minutes. When movements are present, vision is rarely more than one-sixth of the normal.

Table III.—Frequency of the Disease.

	Per cent.
Romiée (1) and Nuel (4)	20
Dransart and Famechon (5)	10
Court (1890) (6)	34·75 of coal cutters.
Stassen (3)	21·7
Libert (7)	23·9
Rogers (8)	17·5

All these figures are obtained by systematic examination of several thousands of workmen.

My figures refer not to the total number of cases of nystagmus, but to the number of men who were compelled to give up underground work for a time.

Table IV.

District.	Cases.	Underground workmen.
A	425	133,000
B	413	56,580
C	27	8,000
D	11	28,000
E	75	12,500
F	2	2,870
G	1	6,855
H	No known cases	5,437

G and H are naked-light districts, F is an anthracite district. B, C, D are almost entirely safety-light districts in which a considerable amount of holing is done. A is largely a safety-light district in which holing is the exception.

I am obliged to Dr. S. W. Plummer, of Durham, for notes of 10 cases which have come under his notice as certifying surgeon for his district. Dr. Plummer is unable to give me the proportion this number bears to the number of men employed, but as the cases are spread over a year the amount of nystagmus, at present, in the North of England appears to be small.

In the Blue books (ref. 9, 10, 11) on "Statistics of Compensation," the number of cases of nystagmus receiving compensation for the years 1910, 1909, 1908, are given as 1618, 1011, and 460. The great increase should be noted, and I believe it will be maintained for some years.

The following is the result of a systematic examination of 110 officials (chiefly firemen) :—Nystagmus was found in 35 cases, eight of which were marked; 11 cases were doubtful; 64 cases were quite free. The visual acuity was tested in all cases, and even when 6/6 was reached the answers were, in many cases, given in a hesitating manner :—

	Nystagmus.	Doubtful.	Free.
Number	35	11	64
Mean age	42·3	39·8	37·1
Mean years of underground life	30	26·4	21·9
Vision—			
6/6—6/9	14	5	48
6/12 or less	21	6	14
Error of refraction—			
Hypermetropia	8	1	4
Astigmatism	8	2	4
Myopia	1	1	4
Degree of error—			
Less than 1 D	12	2	4
1 D and over	5	2	8
Colour of hair—			
Fair	12	2	6
Medium	9	4	13
Dark	14	5	45
Colour of eyes—			
Blue and grey	22	3	29
Light and dark brown	13	7	35

Cost of the Disease.

I have exact figures of the amount paid to 425 cases of nystagmus during one year. Taking this figure as a basis then the cost of the 1618 cases in the kingdom would be £31,853 in compensation for one year. That this figure is a reasonable one may be seen from the following table compiled from the Blue books quoted above (10, 11, 12) :—

Table V.

Year.	No. of cases.	Increase.	Cost of all industrial diseases.	Increase.
		per cent.	£	Per cent.
1908	460	—	13,382	—
1909	1011	120	27,288	104
1910	1618	60	42,507	55

It will be seen that the increased cost of charges due to industrial diseases bears a similar rate of increase to that shown by nystagmus.

In addition to the compensation charges the employers lose the profit of the work done by these men, while the workmen lose a sum in wages which may well be double the sum quoted. In many cases the men are not able to earn as much during the 12 months preceding their failure to work.

Taking all these factors into consideration I estimate that there is a loss to the kingdom of at least £100,000 a year due to nystagmus.

Table VI.—Disability.

Back at old work	59 (43 latent)
Idle	216 (average length of idleness to date, 10·4 months)
Surface work	124 (average time spent on surface to date, 13·3 months)
Dead	1

Twenty-four cases tried to work underground and failed.

This table presents too dark a view for the following reason. My cases are taken from all over the country, and in many places it was only the worst cases that I saw.

Historical Account of Miners' Nystagmus and Theories of its Causation.

The first case was discovered by Decondé (12) in 1861. C. Bell Taylor (13) published a paper in 1875 on "Miners' Nystagmus, a New Disease," Alf. Graefe, 1873, Nieden (14), 1873, Dransart (15), 1877, were amongst the earliest observers. The chief workers since that date have been Snell (16), Court, Jeaffreson (18), and Reid (19), in England; Romiée and Nuel in Belgium; fellow workers with Nieden and Dransart in France and Germany.

Romiée points out (1) that the Davy lamp was recommended for use in 1851 (10 years before the first recorded case), and states that after the compulsory use of the Mueseler lamp in Belgium in 1876 the cases of nystagmus became more numerous. He says that our forefathers were at least as good observers as we are, and that if the disease had existed then they would have discovered it.

There are two chief schools with regard to the etiology of nystagmus. One, of which Romiée, Court, and Thompson (20) are the chief exponents, attributes the disease to the strain of excessive accommodation, the result of deficient light. The second school, to which Snell, Dransart, Nieden, and Nuel belong, attributes the disease chiefly to the position assumed by the collier, and thinks that there is a local myopathy affecting the elevator muscles of the eye.

Romiée brings forward very strong evidence in support of his theory. He quotes figures which show that with improvement of the lamps used there is a great diminution in the percentage of nystagmus. After the introduction of the powerful Wolf lamp in 1908 the cases of nystagmus at one pit fell 50 per cent. below the figures of the same pit in 1891.

He quotes Lewillon of Mons ((1) p. 84)—

Percentage of cases with the Mueseler lamp	38
„ „ electric lamp.........	19

Court (6) gives the following figures as result of a systematic examination—

597 men from a safety-light pit	207 cases of nystagmus.
572 „ naked „	32 „ „

Of these 32 cases, 29 had worked in a safety-light pit at one time.

Libert (7):—

825 hewers with Narsaut or Mueseler lamp	35·3 per cent.
316 „ benzine lamp	24·4 „

Stassen (3), on the other hand, says that the number of cases is almost as great in a naked-light pit as in a safety-light pit.

The alternative theory is that position has the predominating influence.

Snell (17), in particular, says that holing* is responsible for almost all cases. He says that the direction of regard is upwards, and that a great strain is thrown on the elevator muscles of the eye. In my experience, a man when he holes looks directly forward, striking the coal at the level of his eyes.

Reid (19) and Jeaffreson (18) say that the condition is due to a disassociation of the movements of the head and eyes and the strain of working in a bad light. Rutten (21), who expresses the current German idea, says that it is due to a compensatory movement of the eyes set up to oppose any inclination of the head or body from the normal ("Gegenrollung"). Peters (22) suggests that the affection is due to affection of the inner ear (labyrinth); Elworthy (23), that it is due to absence of colour and corresponding retinal fatigue.

Conditions Determining the Occurrence of Miners' Nystagmus.

My chief aim, up to the present, has been to obtain statistical and other evidence as to the conditions which obtain in work, with a view to pointing out the way to prevention; and the 400 cases investigated have been analysed with this end in view.

---

* A method which the collier sometimes uses to win his coal. The coal is undercut a distance of 3 or more feet and then wedged down from the top. The miner in many cases has to lie on his side, and often drags his body under the ledge he has cut.

Table VII.—Age and Duration of Underground Work.

Average age at onset	40 years.
„ period of underground life before onset	26·96 years.
„ interval between onset and failure	11·14 months.

Occupation.

In this table figures are given as to the percentages of cases and of men employed in various ways in a steam-coal colliery. The latter data are taken from two Welsh steam-coal collieries employing over 2,500 men.

Table VIII.

Occupation.	No. of cases.	Per cent.	Per cent. of workmen in steam-coal pit.
Colliers and collier boys	337	84·25	60
Timbermen and repairers	29	7·25	8·7
Hauliers and haulage men	24	6	14·6
Rippers	3	0·75	2
Contractors and hard ground men	2	0·5	7·5
Labourers	3	0·75	1·8
Under officials (firemen)	1	0·25	1·9
Hitcher	1	0·5	
			3·5 various

It will be seen that the men who work at the coal face are the class chiefly affected, but that no class of worker is exempt. It is interesting to note that the first two classes contain 91·5 per cent. of the cases. These men are the most skilled workers, and have to use their eyes more than the others.

The collier places each blow of his mandril accurately, or loses so much work. I have tested colliers by making a mark, the size of a half crown, on the coal with chalk. Every collier struck the mark accurately with the pick when I asked him to do so. The timbermen have to adjust accurately the pieces of timber used to support the roof, and the work is of a very skilled nature.

A few words on the manner in which the collier obtains his coal are necessary. There are, roughly speaking, three ways—

(1) By holing.

(2) By taking advantage of the cleavage lines of the coal.

(3) By the use of mechanical coal cutters.

(1) Holing has been briefly described above. In thin seams, or when the coal is undercut beyond 3 feet, the collier has to assume a reclining position. In thicker seams the collier often kneels at his work, and then his vision is directed downwards.

(2) In thick seams, or where the pressure is great, one edge of the coal is freed and the coal forced out with a bar. In comparison with the last method little skill is required, except in the care of the roof.

(3) Coal cutters. Here the men are more like labourers and not skilled workmen.

Table IX.*

Much holing	111	209
Some „	98	
Little „	22	129
No „	107	

Table IXa.

Of the 111 cases holing was done :—

On side	77
On knees	25
With body inclined	9

* This table applies to colliers only; a few cases where the man had been a collier at one time are added.

Table X.—Figures given by Returns made from 43 Collieries (one district).

	Collieries.	Percentage of nystagmus.
Much holing	30	0·69
Little or no holing	13	0·79

Thickness of Seams.

The thickness of seam does not appear to be of great importance, but as I am unable to give the relative proportion of men working in the several seams my figures are not so valuable as they might be.

Table XI.—Thickness of Seams.

Less than 2 feet	1
2—3 feet	43
3—4 „	85
4—5 „	102
5—6 „	50
6 feet and over	72

Three men had worked with coal cutters.

Table XII.—Returns from 43 Collieries.

Seam.	No. of collieries.	Percentage of nystagmus.
Less than 3 feet	16	0·81
3—4 feet	14	0·54
4—5 „	14	0·80
5—6 „	5	0·58
Over 6 feet	6	0·41

In this table some of the collieries, having more than one seam, appear more than once. The thinnest seam I visited was at Radstock, near Bath, through the courtesy of Mr. G. McMurtrie. The seam was from 12 to 14 inches thick, and all work was done on the side. The pit was lit with candles and no case of nystagmus had been heard of in the district. I examined men at the face. All told me they had no trouble with their eyes and that they had never heard of the disease. There was one very slight case of nystagmus in a man who had worked in a steam-coal pit, but who assured me he had never had any trouble with his eyes.

Light.

The great difference between a coal pit and a metalliferous mine is that in the coal mine a safety lamp is often required. In the Cornish tin mines candles are used and no nystagmus is present.

In reply to a letter of mine Dr. J. Telfer Thomas kindly sent the following: "I have been in practice in the mining district of Cornwall for the past 23 years and have never seen a case of miners' nystagmus there. . . . . It does not attack tin miners."

There is a great difference between the light given by a candle and that from a safety light. The candle gives more light, remains constant through the day, throws no shadows, and, most important of all, can be placed very much nearer the coal face. Some miners place the candle under the ledge of coal they are undercutting. The safety lamp gives less light, quickly becomes dirty, throws shadows, and must be placed out of reach of the pick. The bonnet and oil reservoir cut off much of the light and produce an area of darkness above and below the lamp. (Court lays stress on all these points.) My figures show as far as I am able to do so the relative percentages between the two classes of pits.

In Somersetshire and the Forest of Dean nystagmus is practically unknown. Both are naked-light districts. Through the courtesy of the Home Office I am able to give the figures for Scotland apart from those of the rest of the Kingdom.

I have made a table from these figures and from the Blue books (30, 31).

Table XIII.

	Scotland.	Rest of Kingdom.
No. of cases	55	1563
Percentage of cases to men underground	0·05	0·21
Percentage of safety lamps used (31)......	28·2	91·6

Nystagmus is four times more common in England and Wales and lamps are used more than three times more frequently than in Scotland. (All the Scottish cases may have come from safety-light pits.)

Table XIV.—(My Figures.)

	No.	Latent.	Receiving compensation.
Safety-light pits.........	392	38	354
Naked-light pits	8	5	3

I have drawn up another table in which I have separated my local cases from the rest. My complete figures represent a good average of the whole of England and Wales.

Table XV.

	England and Wales.		Rhymney Valley.	
	Men employed.	Percentage of nystagmus.	Men employed.	Percentage of nystagmus.
Naked lights	8·4 or 1	0·89 or 1	12 or 1	2·66 or 1
Safety	81·6 9·7	99·01 111·2	88 7·5	97·34 36·5

In England and Wales the percentage is 1 to 12·5, in the Rhymney Valley 1 to 5. Of the eight cases from house-coal or naked-light pits five had worked in safety-light pits; five were unaware of the fact that they had nystagmus.

Of the three cases who were bad enough to fail, one was so deaf that I could get no history, one had worked seven years with safety lamps, and the third (Case 6) developed nystagmus immediately after the shock following the Darran explosion, in which he was one of the survivors. He had no trouble with his eyes before.

Murton Colliery, Sunderland, is a very large colliery, and the only one in England where electric lamps have been extensively used for years. In reply to a letter of mine asking for information, Mr. Wood sent me the following:—"*Nystagmus.*—Our cases have been so few and far between that I have no information of value to give you." No comment is necessary.

In one district which I visited the men complained to me that the oil used in a certain colliery was very poor in lighting quality. I saw the certifying surgeon of that district, and he told me that he had more cases of nystagmus from that small colliery than from all the other collieries around. In another district I had many complaints that the lamps were not well cleaned. Here, again, the percentage was above the average. In several collieries where the average was high, the lamps were cleaned by hand, a very inefficient proceeding compared with the machine cleaning.

Table XVI.—Oil used in 43 Collieries.

	No. of pits.	Percentage of nystagmus.
High grade paraffin	16 (14 entirely)	0·58
Mixture of colza and paraffin	4 (3 ,,)	0·68
Spirit or naphtha	6 (5 ,,)	0·71
Mineral colza	15 (14 ,,)	0·81
Colza	9 (8 ,,)	0·93

Lamps compared with the standard candle (Report of Mines Accident Commission, 24):—

Davy	0·19
Clanny	0·62
Mueseler	0·69
Marsaut	0·68

We do not realise how much of our light is due to diffused reflecting power of the walls of any building we happen to be in. In the coal mine practically all the light is absorbed.

Ocular Defects.

This introduces the personal factor of the disease, and one which has been too much neglected in the past. It has always struck me as strange that when two men are working together under similar circumstances one may be affected and the other left. I have examined men who have been underground for 60 years without showing any sign of nystagmus. Yet a case may develop after six months' underground life (Case 19).

The disease has been shown to attack the men who use their eyes the most in a much larger proportion than the other men.

My figures are incomplete, but they show that a large proportion of men suffer from error of refraction. T. Thompson (20) brings out this point, but his cases are few. Romiée also says (2) that a large proportion are hypermetropics, but in his later communications does not lay much stress on this point. Dransart (5) and Snell (17) say that the majority of cases are normal.

I have tested my cases for visual acuity and error of refraction; it was impossible to examine all, from lack of opportunity or time. As it was important not to call the attention of the latent cases to the fact that they had nystagmus these were not examined.

Table XVII.—Visual Acuity.

Too bad to test	18
Not examined	39
Normal	43
6/9	53
6/12	46
6/18	67
6/24	52
6/36	43
6/60	39

The fraction gives the proportion of power of vision to normal; thus 6/12 = half normal, etc.

Table XVIII.—Showing Error of Refraction.

Not examined	46
Latent	43
Too bad to test	31
	120
Apparently normal	18 (6·4 per cent.)
Error	262 (93·6 per cent.)

Table XIX.—Showing Kind of Error.

Hypermetropia (long sight)	105
Myopia (short sight)	37
Astigmatism—	
Hypermetropic	91
Myopic	26
Mixed	3

Table XX.—Showing Degree of Error.

Less than 1 Diopter	102 (chiefly astigmatic)
1 to 2 D	110
2 to 3	30
3 to 4	9
4 and over	11

All have weakness of accommodation. They are unable to fix a point for any length of time, and they turn away their heads or cover their eyes with their hands after a short time.

Many cases show internal squint, and injection of the eyes is frequent.

Observations on Pigmentation.

Early in my investigation I noticed that several of my severe cases had blue eyes and fair hair. The possibility of a partial albinism suggested itself, and I took notes of all cases. Nettleship (25), in his Bowman lecture and elsewhere (26), suggests that congenital nystagmus may be due to a partial albinism confined to the eyes.

Table XXI.—Showing Colour of Hair and Eyes in Nystagmic Cases. With each colour eye three sets of figures are given; the first refers to fair-, the second to medium-, and the third to dark-haired people.

Blue	62, 61, 47, eyes only noted, 15, total 185	258, or 64·5 per cent.
Blue-grey and grey	15, 25, 32 „ 1, „ 73	
Light brown	6, 13, 17 36	142, or 35·5 per cent.
Dark brown	3, 43, 53, eyes only noted, 7, total 106	

Table XXII.—Control Test, Adult Miners, same District.

Blue	59, 76, 57, total 192	272, or 41·97 per cent.
Blue-grey and grey	13, 17, 50, „ 80	
Light brown	5, 70, 43, „ 118	376, or 58·03 „
Dark brown	5, 53, 200, „ 258	

Table XXIII.—Colour of Hair.

	Nystagmus.		Control.	
Fair	86	228	82	298
Medium	142		216	
Dark	149		350	

Relation of Nystagmus to Accident.

In 76 cases there was a distinct connection between the onset of the disease and an accident. The accident was to the head in 33 cases, to the eyes in 28, and to the body in 15. I have no space at present to quote cases. I am also convinced that nystagmus predisposes to accident, but the subject is such a difficult one that I am unable at present to give any figures.

Diagnosis of Nystagmus.

The patient should be asked to fix the finger tip held about 12 inches in front of and on a level with the eyes. If movement does not appear, gradually elevate the finger, asking the patient to follow. Note the degree above the horizontal at which movement begins; this gives a rough test of the severity of the disease (Romiée (1)).

If this fails ask the patient to stoop or exert himself. The eyes may be examined in a mirror held under the patient's head while he remains in a stooping position. A pencil of light may be focussed at the junction of the cornea and sclerotic in a dark room.

The rotation test advised by Reid (19) should not be used, as normal people give the test. I have found the ophthalmoscope of no assistance. Several examinations may be necessary.

A test which is valuable, although not absolutely conclusive, is the following " head test." If the patient is asked to look up while the head is kept strongly flexed by the observer's hand a head tremor may often be felt in cases where the movement of the eyes is absent. This test is frequently given by long-standing cases in which movement has disappeared.

When movement occurs the character must be noted, as true miners' nystagmus gives a rotatory movement. If the movement is purely lateral and is not equal on both sides grave suspicions should be aroused. The case may be one belonging to the group of nervous diseases, such as disseminated sclerosis, syringomyelia, or cerebellar tumour, which give the symptom of nystagmus.

It is also necessary to exclude cases of high myopia, of which I have seen two examples, and the congenital conditions, such as hereditary nystagmus or albinism, of which I have seen several examples. Lastly, some people can produce nystagmus voluntarily (27, 28). I have seen one case.

Prognosis.

Slight cases recover quickly and completely; severe cases slowly and often incompletely. Table VI, containing so many bad cases, is not so just as the following, taken from the three large colliery companies for a period of four years:—

Back at old work	57
Left employ = recovery	5
Surface work (including six who failed underground)	68
Still idle	30

The prognosis in individual cases depends on the age, length of symptoms before failure, degree of elevation necessary to produce movement, visual acuity, presence of error of refraction and the presence or absence of tremor of the head.

Causes of Nystagmus.

I regard the disease as one of extreme complexity and one in which many factors are at work. The chief factor is strain caused by deficient light. Error of refraction, deficiency of retinal pigment, position assumed at work, accident and ill-health are all factors of less importance.

As the result of working for long periods in the comparative darkness of the pit, the cells of the retina probably lose their power of producing sufficient pigment for exact vision. This failure occurs sooner in fair blue-eyed people and in people who, owing to refractive error, are subject to a greater eye-strain. The more frequent occurrence of nystagmus in winter, the loss of visual acuity, the dread of light, are all points in favour of this theory. Another is the oblique position in which the head is held in many cases. This is probably due to an attempt made by the patient to bring a fresh part of the retina into play, the central portion being worn out: "In the dark and with an absolutely homogenous field before them the eyes are always moving" (Maddox, 29). These conditions nearly obtain in a coal mine.

This diminished power of fixation calls for efforts by the higher centres for better vision. Excessive accommodation and resulting eye-strain follows. Darkness itself is not enough to set up nystagmus.

Horses do not show any signs of the disease even after 10 years' continual underground life. I examined 46 horses without result.

Lower animals have no central fixation (Maddox) and the horse has apparently two fields of vision and consequently no need for associated vision. They are also free from eye-strain.

The position assumed by the miner, especially when the head is flexed and the eyes look up (Jeaffreson), also has some influence.

The total result is that the impulses passing to the brain are not so exact as they should be and that the centres governing the associated movements of the eyes are correspondingly disturbed. The intimate connection between these centres is lost and incoördinate movements of the eyeballs result.

Snell, Dransart, and others lay great stress on the fact that nystagmus is best seen and often only brought out when the eyes are elevated, and they attribute this to weakness of the elevator muscles. It is not the muscles which are affected but the movements of elevation.

This failure is most marked in elevation of the eyes for two reasons:—(1) The movements of elevation are the weakest and least often used of all the associated movements of the eyes; when the centres are put to a severe strain they are the first to go. (2) If the movements of the eyes are noticed in the vertical plane it will be seen that in all positions below the horizontal there is an associated convergence, while in all positions above there is a tendency to divergence. The eyes are in a position of maximum stability when depressed and converged, and consequently nystagmus is not found in this position except in severe cases.

In many cases, and in most long-standing cases, the head is thrown back, the eyelid droops, and the patient looks out at you from half-closed eyes. He assumes this position, in my opinion, to bring his eyes into a relative position of depression and convergence (the position of maximum stability), and at the same time to direct his plane of regard horizontally forward.

The character of the movements, the sudden onset in many cases (50), the influence of accident, and the character of the tests used to bring out the movements, all point to incoördination as the starting point of the disease.

Preventive Measures.

The results of this investigation show clearly that the most important preventive measure is improvement in the lighting power of the miner's lamp. If oil lamps are used, careful attention should be given to the cleaning of the lamps and quality of oil. If possible, a lamp giving a diffused light should be introduced. Shades should always be fitted to the lamps to be used in going to and from the face. (This is now in force in the Powell Duffryn pits of the Rhymney Valley.)

No man with error of refraction should be allowed to work underground. Simple tests should be put to all fresh men by the managers, and if the result is not satisfactory, the man should not be employed without a medical examination. I believe that if this precaution were taken, half the cases of nystagmus could be avoided. The introduction of coal-cutting machines in thin seams is also of assistance.

Table XXIV.—Use of Coal Cutters (43 Collieries).

	Collieries.	Percentage of nystagmus.
Extensively	6	0·49
Partly	15	0·73
Not used	22	0·81

Improvement of ventilation, which also means a better light, is also of the greatest importance, since the light given by a lamp falls off very rapidly as the oxygen percentage of the air diminishes.

I am now engaged in the measurement of illumination at the working places, in experiments on the effects of introducing electric lamps, and in the collection of further evidence as to the causation of the disease, with a view to a further and much more full report.

In presenting this preliminary report, I wish to acknowledge the very great and willing help I have received from both the mining and the medical fraternities. To Dr. Haldane, especially, I am indebted for much valuable help and encouragement.

The Directors of the Powell Duffryn Company and Rhymney Iron Company, the South Wales Colliery Owners, and the South and West Yorkshire Colliery Owners, have given me every facility.

I should like to thank, in particular, Mr. W. E. Garforth, Mr. Binns, of Derby; Dr. Hughes, of Blackwood; Mr. Phillips, of New Tredegar, and Mr. Rutherford, of Rhymney. I have also received help from the following gentlemen :—Dr. Elworthy, Ebbw Vale; Drs. W. and A. Martin, Leighton Davies, of Cardiff; Dr. Hislop, Wrexham; Dr. Leary, Alfreton; Dr. Wood Wakefield; Dr. Buncle, Pontefract; Dr. Nicholson, Leeds; Dr. Symes, Chesterfield; Drs. Kerr and Emerson, of Sheffield; Dr. Gray, Mansfield; Dr. Morris, Mardy; Dr. Robson, Penarth; Drs. Thomas and Phillips, Ystrad; Dr. Morris, Tylorstown; Dr. Richards, Risca; Dr. Astbury, Aberaman; and Dr. McGhie, of Merthyr Vale.

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(23) Elworthy, 'Brit. Med. Journ.,' 1910, vol. 2.
(24) Boulton, 'Practical Coal Mining.'
(25) Nettleship, 'Trans. Ophth. Soc.,' vol. 29, p. 125.
(26) „ *ibid.*, vol. 31, p. 162.
(27) „ 'Diseases and Injuries of the Eye.'

(28) Mauersberg, 'Ophthalmoscope,' 1910, vol. 8.
(29) Maddox, 'Tests and Studies of the Ocular Muscles,' 1907, 2nd edition.
(30) 'Reports of His Majesty's Inspectors of Mines,' 1910, Cd. 5794, Cd. 5676, Cd. 5676, 1 to 7.
(31) Debate on Coal Mines Bill, reported in 'The Times,' November 24, 1911, and in the 'Colliery Guardian,' December 1, 1911, p. 1075.

On the Effects of Castration and Ovariotomy upon Sheep.

By F. H. A. Marshall, Fellow of Christ's College, Cambridge, and University Lecturer in Agricultural Physiology.

(Communicated by Prof. J. N. Langley, F.R.S. Received December 20, 1911,—Read February 22, 1912.)

It is well known that castration, when performed in early life and before sexual maturity has been reached, has a marked effect not only in inhibiting the development of the accessory male organs, but in changing the general conformation of the body. Thus in castrated guinea-pigs, oxen, and capons, as well as in eunuchs, the bones of the limbs tend to be abnormally long, this result depending upon an arrest in the ossification of epiphyses. The secondary male characters are also in many cases suppressed, so that there is an apparent approximation to the female type. Thus in red deer if the testes are removed in quite immature animals the antlers fail to make their appearance, and in fallow deer castration at birth limits the horn formation to the development of single dugs. Secondary sexual characters, however, are not always correlated with the presence of the essential reproductive organs, even in mammals. Thus the withers in the gelding resemble those of the horse rather than those of the mare, in which the withers are lower. Moreover, in certain varieties of cattle in Italy, the horns in the ox, if castration has been carried out young, are far longer than those of either the bull or the cow.

Ovariotomy in the female is often said to lead to the assumption of male characters, but there is little experimental evidence that this is actually the case. In the human female complete removal of the ovaries, if carried out in early life, besides preventing the onset of puberty and the occurrence of menstruation, produces effects on the general form and appearance, individuals so operated upon being said, in some cases, to show resemblances to men. Abnormalities in the ovaries have been described as producing similar results. Thus, Rörig* records three cases in which female deer possessed horns, and were shown on dissection to have had abnormal ovaries. Darwin* states that female deer in old age have been known to acquire horns. R. Wallace* says that in old mares the neck tends to acquire an arch as in the stallion. The occasional growth of hair on the face in old women is a phenomenon of the same kind. Similar observations have been made upon birds, especially ducks, poultry, and game birds. Darwin mentions the case of a duck which, when 10 years old, acquired the plumage of the drake. Other cases are those of hens which in old age assumed secondary male characters and started to crow. Hunter* mentions a hen pheasant which had male plumage associated with an abnormal ovary. Numerous other instances have been described, but it is not apparent that such an acquirement of male characters by female individuals is always correlated with an abnormality in the reproductive organs. According to Gurney† the assumption of male plumage is generally associated with sterility in female gallinaceous birds, but not, as a rule, in female passerine birds. Thus Gurney describes a hen chaffinch with male plumage and an unlaid egg, and a hen redstart with male plumage and a number of developing eggs.

Guthrie‡ has described an experiment in which the ovary was removed from a fowl. After the removal an ovary from another fowl was engrafted, but the transplanted ovary underwent degeneration. "The pullet acquired not only the outward anatomical features of a cock—cock's comb, wattle, long hackle and tail feathers, rapidly developing spurs, carriage, etc., but the behaviour as well was that of a male; it exhibited the pugnacious attitude towards other cocks, was attracted by hens, and even went so far as to tread hens as a cock."

Goodale§ records two experiments in which the ovaries were removed from ducks. One was operated upon in the early spring of 1909 and the other in August, and both were still alive at the time of writing (November, 1910). The first bird had only undergone a slight change in plumage, but the second made a definite though gradual advance towards the male type. This bird is described as having come closely to resemble the drake in summer plumage, possessing brilliant green feathers on the head, a white neck ring, much claret colour on the breast, and other characters which are typically male. The voice, however, still remained that of the female, and the colour of the bill and some of the feathers also continued unchanged. Goodale suggests that the bird in question in course of time may assume the complete secondary sexual characters of the drake.

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* For references see Marshall, 'The Physiology of Reproduction,' London, 1910.

† Gurney, "On the Occasional Assumption of Male Plumage by Female Birds," 'Ibis,' 1888, vol. 6 (5th series).

‡ Guthrie, "Survival of Engrafted Tissues," 'Journ. Exper. Med.,' 1910, vol. 12.

§ Goodale, "Some Results of Castration in Ducks," 'Biol. Bull.,' 1910, vol. 20.

Apart from these cases there appears to be no definite experimental evidence, at least from among vertebrates, of the assumption of male characters as a result of ovariotomy.

Observations and Experiments upon Sheep.

The various breeds of sheep differ considerably in regard to horn growth. Some are horned in both sexes, though the degree of development of the horns varies according to sex (whether ram, wether, or ewe); some are hornless in both sexes, while others, again, are horned in the male, but hornless in the female. At the Royal Agricultural Society's Show, at Norwich, last summer, I made some observations upon representatives of most of the British breeds. Among those breeds which are ordinarily described as hornless, the males of the following in many cases had rudimentary horns or "scurs":—Hampshire Down, Suffolk, Lincoln, Leicester, Derbyshire Gritstone, Cotswold, Devon Longwool, Dartmoor, and Cheviot. (The rams of the last-mentioned breed had particularly large scurs, which almost amounted to horns in some individuals.) On the other hand, the ewes of these breeds in no case had any scurs. Wethers were not represented at the Norwich Show, but it would seem from other observations and enquiries relating to some of the above-mentioned breeds that the scurs do not generally appear in the rams until they are more than a year old, and that the wethers do not grow them at all.

In those breeds which are horned in both sexes the horns of the ewes are less perfectly developed than those of the rams. Thus, in Dorset Horns, the ewes' horns are finer and lighter than the tups', and, as a rule, do not curl so much. The wethers' horns are either more or less intermediate, or resemble those of the ewes. Dorset ewes are born without horns, but the ram lambs generally have incipient horns which are visible at birth. It is noteworthy that the horns of the wethers are most like those of the ewes when they are castrated early, and that postponement in castration diminishes the resemblance.* In the Lonk sheep the ewe and wether are not unlike in their horns, but those of the wether are rather more contracted and grow closer to the head. The rams' horns are much better developed.* The Scotch Blackfaced sheep are similar.†

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* These statements are based on personal observation or on information obtained from breeders in the Isle of Wight.

With Herdwicks, which are the breed used in the investigation described below, the rams have large coiled horns and the ewes are hornless. It must be mentioned, however, that at the Felldale Association Show last year, at Eskdale, a very small proportion of the Herdwick rams present were without horns, but this condition is very unusual. Moreover, according to information given by Mr. John Rothery, of Wastdale Head, Westmorland, the ewes may very occasionally have scurs, though their occurrence is far less frequent than formerly. The ewes at the Norwich Show were without any sort of scurs, but the rams were typically horned. Mr. Rothery informs me further that Herdwick ram lambs are, as a rule, castrated when six weeks old, and do not then grow horns, but if the testes are removed at a later age, horns with some degree of development are present. The skull of the Herdwick wether has been described by Shattock and Seligmann,‡ who say that it resembles the skull of the ewe in general configuration, being less rugged than the skull of the ram, while the bones are thinner and the cranial cavity does not extend so far forward as in the ram.

Two Herdwick ram lambs, born in the late spring, were sent to Cambridge from Westmorland in August, 1910. Each of these had horns between 2 and 3 inches in length. One of these was kept as a control, and now possesses large coiled well-developed horns. The other was castrated when about three months old. In the case of the latter the horns ceased to grow, and in the following spring became loose (apparently as a consequence of being butted by the ram), and eventually came off, leaving low rounded knobs, representing the horn cores. The wether is still alive, but no new horns have developed.

Two more Herdwick ram lambs, born in May, 1911, were castrated in the following autumn, one when about four months, the other when about five months old. In the former case the horns were about $4\frac{1}{4}$ inches long, and have since remained stationary. In the latter case the horns were 6 inches long, and likewise have since ceased to grow. In another lamb, which has not (as yet at any rate) been castrated, the horns are now (December, 1911) 11 inches long.

It would appear, therefore, that not only does the development of horns in

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* I am indebted to Mr. W. Ralph Peel for a series of photographs illustrating horn growth in Lonk rams, wethers, and ewes of various ages.

† It has been thought worth while to relate the facts mentioned above since they do not appear to be recorded in any of the literature on sheep.

‡ Shattock and Seligmann, "Observations upon the Acquirement of Secondary Sexual Characters," 'Roy. Soc. Proc.,' 1904, vol. 73.

Herdwick rams depend for its initiation upon a stimulus set up by the testes, but that this stimulus is necessary for the continuance of horn growth, which stops as soon as the testes are removed.

Six Herdwick ewe lambs were also obtained in August, 1910, and three of these were operated upon when about three months old, the remainder being kept as controls. At that time none of these six ewes had any perceptible trace of horns or scurs. The operations and subsequent history of the ewes were as follows :—

(1) The ovaries, Fallopian tubes, and uterus were completely removed. About seven months after the operation, when the ewe was 10 months old, it was noticed that small scurs were beginning to grow from the frontal bones. These continued to enlarge, but only very slightly. When the ewe was about 17 months old it died from hoven. The skull and skeleton were kept and preparations made. The scurs may now be seen on the skull as small projections about ⅛ inch long. The epiphyses of some of the bones (particularly those of the metacarpal and metatarsal bones) appear to be slightly longer than those from the skeleton of a normal Herdwick ewe of the same age, and this result may possibly have been due to the extirpation of the ovaries.

(2) The ovaries and Fallopian tubes (but not the uterus) were removed. The ewe is still alive. There are at present only doubtful traces of scurs, and these, such as they are, have made no progress in development in the last seven months, but seem rather to have become reduced so as now to be scarcely perceptible.

(3) The ovaries, Fallopian tubes, and upper parts of the uterine cornua were removed. The ewe is still alive, but, as in the preceding experiment, there are now only doubtful indications of scur growth, the skull being merely somewhat bumpy in the position where the horns or scurs should grow.

In each of the three animals the removed generative organs were quite infantile. They have been preserved.

It is clear, therefore, that the removal of the ovaries in young Herdwick lambs before puberty does not lead to an acquirement of horns, which in this breed are male characters. It must be mentioned, however, that in neither of the three normal ewes could any trace of scurs be detected. One of these was killed at about the same time as the death of the spayed ewe (No. 1) and the skeleton of this animal was preserved. The skull is quite smooth in the position corresponding to that of the scurs in the spayed ewe.*

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* The other two normal ewes unfortunately died earlier, and their skulls were not kept, but no scurs had been detected in these shortly before their death.

It is just possible, therefore, that the development of scurs in the first ewe and their doubtful development in the two ewes that are still alive, may have been due to the removal of the ovaries, which on this view may be regarded as organs exercising an inhibitory influence on horn growth. In other breeds (*e.g.* Suffolks), where scurs frequently grow in the ram, these do not generally (if ever) make their appearance until the second year, and it is conceivable that in the two spayed ewes which are still being kept, definite scurs may manifest themselves at a later age, though this seems unlikely, since the animals are now 19 months old. (Compare, however, Goodale's duck, referred to above.) It has already been mentioned that, according to information supplied me, scurs occasionally grow in Herdwick ewes. In view of this fact it would appear that the only conclusive way of determining definitely whether scurs can be induced to grow in Herdwick ewes as a result of ovariotomy would be to spay a considerable number of individuals and compare these with an equal number of unspayed sheep. Lastly, it is always possible that the presence of the ovaries in early life, or even before birth, may exercise a permanently inhibitory influence which can last after the operation of removal.

Conclusions.

(1) The development of horns in the males of a breed of sheep in which well marked secondary sexual differentiation occurs (as manifested especially by presence or absence of horns) depends upon a stimulus arising in the testes, and this stimulus is essential not merely for the initiation of the horn growth but for its continuance, the horns ceasing to grow whenever the testes are removed.

(2) The removal of the ovaries from young ewes belonging to such a breed does not lead to the development of definitely male characters, except possibly in an extremely minor degree.

The work was carried out at the Field Laboratories, Milton Road, Cambridge. The expenses were partly defrayed by a grant from the Government Grant Committee of the Royal Society, to whom I am under obligations. I wish also to express my indebtedness to Mr. G. A. Banham and Mr. J. G. Runciman for their kind assistance in carrying out the experiments.

The Stomatograph.

By W. Lawrence Balls, M.A., Fellow of St. John's College, Cambridge, Membre de l'Institut Egyptien, Botanist to the Egyptian Government Department of Agriculture.

(Communicated by Francis Darwin, F.R.S. Received December 22, 1911,—Read February 22, 1912.)

Introduction.

The appliance here described was the result of an attempt to devise a self-recording modification of Mr. Francis Darwin's "Porometer."*

This modification was designated the "Stomatograph," since it automatically records the changes which take place in the mean stomatal aperture of a given leaf, by measuring the velocity at which air under constant pressure can be passed through the tissues. Certain special mechanical difficulties had to be overcome in order to employ it for researches on stomatal movement in the open field, with particular regard to the cotton plant in Egypt.

The following article gives a brief account of the evolution of the appliance ; a description of a "stomatograph" as made by the writer, with specimens of records actually obtained from it ; a short discussion of the sources of error, indicating the modifications which I have suggested with the aim of making it into a precise and sensitive air-pump.

§ 1. *Development of the Appliance.*

The alarming deterioration of the Egyptian cotton crop, produced mainly† by unsuitable soil-water conditions, made it imperative to obtain some precise knowledge of the relationship of the plant to water. The stomata being of pre-eminent importance‡ in this respect, an investigation of their behaviour in the field crop appeared to be the first line of attack. Such an investigation was beyond the powers of our laboratory§ unless self-recording apparatus could be charged with the task. There was no existing appliance for the purpose, except the indirect method by means of leaf temperature,|| and this was quite unsuitable for field work, owing to the much greater

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* Darwin, F., and Pertz, D. F. M., 'Roy. Soc. Proc.,' 1911.

† 'Report of Egyptian Government Cotton Commission,' 1910.

‡ See, however, Lloyd, F. E., 'Carnegie Inst.,' 1908.

§ Department of Agriculture, P.W.M., Cairo.

|| Darwin, F., 'Bot. Gaz.,' 1904.

magnitude of the temperature-changes produced by other causes,* such even as clouds by night.

The horn hygroscope and the cobalt-paper method were incapable of adaptation to automatic performance, but just as it seemed that there could be no alternative to personal observation, Mr. Francis Darwin showed the writer his Porometer. This simple appliance solved the difficulty.

The writer's first recording porometer consisted of a constant pressure aspirator attached to the porometer chamber; the out-flowing water from this was collected in a syphon-bucket, supported on a spring and carrying a stylus. The slow descent of the bucket, followed by its sudden rise when emptied by the syphon, marked on a clock drum the time elapsing during the passage of a certain volume of air. Apart from some irregularity in the discharge of the syphon, this appliance was useless for field-work, since the aspirator acted as an air-thermometer, and the discharge of water was consequently irregular, even though the porosity of the inlet were constant. Certain data were obtained with it in the laboratory, and during limited periods of time in the field, which indicated the details to be considered.

A suggestion made by Mr. F. Hughes† led at this stage to the trial of a method in which a gas-holder was continually being charged with air-bubbles from a small water pump. The altitude of the gas-holder varied with the aperture of the exit, viz., the stomata, and an integrated pressure-graph was thus obtained. This method was not developed further, owing to the satisfactory performances of the appliance next described, which was more compact and portable.

§ 2. *The Stomatograph.*

Retaining the principle of the porometer, with modifications in the method of attaching the chamber to the leaf, an electrically-driven air-pump was constructed, which forced a fixed volume of air at constant pressure through the leaf at each stroke, and recorded the time taken in so doing. It was then easy to record the changes which were taking place in the porosity of the leaf which sealed the exit from the pump.

By arranging the pressure and pump capacity suitably, the stroke of the pump was made of sufficient frequency to prevent any notable error being caused by variations in temperature or barometric pressure during the occupation of the pump by any one charge of air.

The cotton plant possesses from 200 to 300 stomata on its lower leaf-surface, and about 100 on the upper, to each square millimetre. The aperture of these stomata, which are of moderate dimensions, varies from complete closure to widest distension during the twenty-four hours. Consequently, the flow of air from one side of the leaf to the other is extremely facile at certain times, and only a very low pressure is required. It was found that a pump discharging 5 c.c. of air at each stroke, under a pressure of 0·5 mm.

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* Balls, W. Lawrence, 'Cairo Sci. Journ.,' 1911.

† Chemist to the Department of Agriculture, Cairo.

of mercury, through a circular area of leaf tissue of 5 mm. radius, was most convenient to employ; with these arbitrary constants, the time occupied in one stroke of the pump varied between extremes of 5 seconds and 50 minutes, according to the condition of the leaf. The pump had to show a maximum variation of not more than 5 per cent. in its capacity per stroke, and in the pressure exerted during the stroke. It had further to be constructed in such a manner as to be thoroughly protected, together with its recording drum, from wind, dust, and insects, when left in the midst of a field of cotton plants. Lastly, its dimensions had to be such that it should be easily portable, and should not damage nor interfere with the surrounding plants. All these requirements have been met by the appliance here figured and described, which is, moreover, very easy to use.

The Stomatograph, as set to fulfil the special purpose for which it was designed, consists of two parts; firstly, a small portable case containing the pump, its operating battery, and a relay; secondly, an electro-magnetic marker, writing on a chronograph, operated over a telegraph-wire by the relay, from a separate battery.

(a) *The Pump* (fig. 1A).—Since the low pressures required made the use of metal faces impracticable, the air was compressed by means of a gas-holder (*G*) floating in a liquid (*L*). This gasholder was suspended by a flexible cord, or metal strip, from the channelled quadrant arm of a short-beam balance (*B*), and counterpoised on the opposite side from a similar quadrant by a soft iron rod (*C*). The pressure was then regulated by adding weights to the gas-holder.

The exit-tube (*Ex*) from the top of the gas-holder was made of fine rubber of 1 mm. bore, fixed at a distance of a few centimetres to the wall of the enclosing box. Thus arranged, it interfered but slightly with the regularity of the gas-holder's movements, though a central vertical tube with three-point guides would be preferable.

The inlet valve which admitted air on the up-stroke of the gas-holder and at the same time defined its maximum content of air, consisted simply of two or three holes (*V*, *V*) in its wall, near the foot. When these holes were brought above the surface of the liquid in the beaker, the air rushed in suddenly, and the consequent sudden diminution in weight was utilised to knock off the electric contact mentioned below.*

To prevent the gas-holder from being jerked upwards too suddenly by the motive power, when taking in a fresh charge of air, a baffle-plate (*Bp*) was fitted to its lower end, which also served to carry the weights which determined the pressure.

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* The insertion of a reversible electromagnetic valve on the exit would allow either positive or negative pressures to be employed, would define the pump-capacity with precision, and would maintain a constant pressure.

Thus arranged, the gas-holder, having been freshly charged with air, and having settled down to temporary equilibrium, sank into the beaker more or

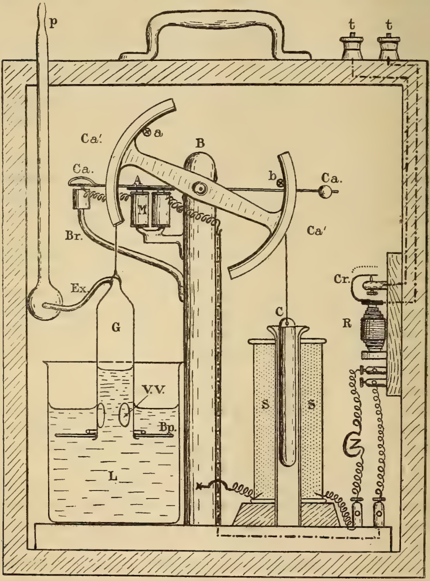


Fig. 1a.

less rapidly according to the aperture of its exit. Having descended to a certain point, with no friction to overcome excepting that of the pin-bearing of the balance, it made an electric contact, which sent a current through a solenoid (*S*, *S*) surrounding the iron rod previously mentioned.

This rod was then drawn down into the solenoid, raising the gas-holder rapidly until a fresh charge of air had been taken in, when the contact was broken. The figure shows the position of the pump at a fraction of a second before the admission of a fresh charge, while the solenoid circuit is still intact.

The use of a solenoid and core in this way has several advantages, the chief of which is that its lifting power is greatest when most required, namely, at the stage shown in the figure. The only other difficulty then left was to provide a prolonged contact, which should be broken when the fresh charge had entered the gas-holder, and not until then. This was effected by a separate light swinging arm (*Ca*) behind the main balance, but on the same pivot, shown also in plan in fig. 1B.

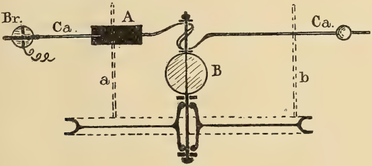


Fig. 1B.

This arm was counterpoised by a sliding weight so as to rest normally at an inclination of 30° to the horizontal (*Ca′*, *Ca′*), the elevated end being that which bore a platinum wire contact. As the gas-holder side of the balance descended, a projecting rod (*a*) which it bore, pressed upon this elevated end, and carried it down also, until the platinum wire came into contact with the platinum-tipped surface of a fixed bracket (*Br*) projecting from the side of the balance-pillar but insulated from the latter. This contact completed the circuit through the solenoid and caused the gas-holder to begin to rise; in order to prevent the contact beam from swinging up immediately from the bracket, and so breaking the contact, a small electro-magnet (*M*) was placed under it, near its pivot, and included in the same circuit; this magnet was wound so as to be only sufficiently powerful to keep the contact arm in contact, by means of a ferro-type armature (*A*).

Contact was thus held until the new charge of air bubbled into the gas-holder, when the sudden diminution in weight of the latter allowed the core to jump further into the solenoid. At this moment a similar projecting rod (*b*) on the core side of the balance-beam engaged with the other end of the contact arm and knocked it off from the holding-down magnet.

The contact being thus broken, the arm swung up into equilibrium, the gas-holder dropped back to temporary equilibrium in the beaker, and a fresh stroke of the pump commenced. A slight alteration in pressure is involved by the necessity for depressing the contact arm, but this is reduced to a negligible amount by making the contact arm as light as possible.

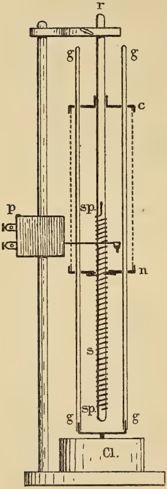


Fig. 2.

Lastly, the connections were arranged as follows: from the battery to the solenoid, then to the metal balance-post, and through its pivot down the contact-arm to its platinum tip. When the circuit had been completed as described above, the current passed into the corresponding platinum wire, which it met at right angles on the insulated end of the bracket, and from there passed by insulated wires into the holding-down magnet, down the side of the balance pillar to the relay (*R*) and back to the battery.

(b) *The Relay.*—This was simply an electro-magnet, wound with thick wire, and attracting an armature against a spring. When the circuit was closed, and the armature drawn down to the magnet, a contact (*Cr.*) was made by the armature, which closed the separate circuit of the telegraph recorder. When short lengths of telegraph wire are employed, as when working indoors, the relay can be omitted.

(c) *The Battery.*—This consisted of three "W.-O." medium cells, which worked the appliance almost continuously for over two months.

(d) *The Case.*—The box containing pump, relay, and battery, was 30 cm. high, and 20 cm. in width and depth. It was provided with a dust-proof door in front, a handle for transport, and bore on its top two binding-screws (*t, t*) from the relay, for the telegraph wire, together with a glass nozzle (*p*) leading from the exit tube of the pump. When placed on the ground beneath the plant to be examined, care was taken to level it approximately, so as to avoid friction of the core on the glass solenoid-lining.

(e) *The Recording Appliances* (*Fig.* 2).—These were placed in the laboratory

50 metres away, and connected with the binding-screws from the relay by double flexible wiring.

The Marker.—This (*p*) was simply a single-beat electric bell, bearing a pen in place of the hammer, and so marking a vertical dash across its trail on the drum whenever the circuit was closed and broken.

The Battery.—A single " W.-O." cell was amply powerful for this circuit.

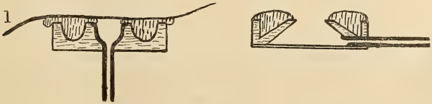
The Drum.—The drum was required to differentiate between marks made at intervals of only 5 seconds, and to record for 30 hours continuously. A vertical chronograph was therefore adopted, descending on a screw, with one rotation of 15 cm. circumference in every hour.

A brief note on its construction, which was remarkably cheap, may perhaps be included. The proper screw was replaced by a spiral (*sp*) of stout brass wire, extended to the requisite pitch, and fitted closely over a glass rod (*r*). The nut (*n*) consisted of three needle-points, chocked to their correct relative heights round a hole in the centre of a piece of tin, which clipped the lower end of the drum ; their points bore on the central glass rod, and their lower sides on the brass wire. With the screw-pitch and weight of drum employed, the clock (*Cl*) was more nearly a brake than a motor.

The hour spindle of the clock was fitted with a double "L" piece, over the upturned ends of which were dropped the lower ends of two vertical glass tubes (*g*, *g*). These passed upwards, parallel with the screw-axis and inside the drum, through two guide holes in the tin nut, and then through two more in the top cover (*c*) of the drum, which was thus free to move vertically down these two driving rods, though constrained to a spiral motion by the screw.

A retort-stand served to hold the axial screw, and a cheap American clock, placed centrally beneath it, drove the drum. The timing was corrected by marking the time at the beginning and end of a record, and cutting the paper off the drum along the hour-line.

(f) *The Chamber.*—Some difficulty has been experienced by workers with the porometer in the cementing of the leaf to the chamber. A method devised by the writer appears to be an improvement, since a tight joint can be made or broken in a few seconds, and may be maintained for several days without direct injury to the leaf. The flange of the chamber (fig. 3A) contains a deep groove, concentric with the chamber itself, this groove being filled to overflowing with paraffin of some melting-point suitable to the particular circumstances, from 50° C. to 30° C. The wax



A Fig. 3. B

is slightly softened by the heat of the hand, and the leaf (l) merely pressed upon it. When the leaf is to be exposed to strong sunlight, a white ring of cardboard is centred over that portion in contact with the wax, at a distance of a millimetre or two, by a three-point support. The chamber employed to obtain the records here reproduced is shown in fig. 3A. On the suggestion of Mr. Francis Darwin this has been modified to fig. 3B, in which the back is made of glass, and so provides a nearly normal illumination to the lower side of the leaf.

When a leaf is to be left for many hours in the open air on such a chamber, it is well to bind it lightly with wool in order to prevent the wind from stripping it off, and so wasting battery power by leaving a free exit from the gas-holder, besides interrupting the record.

§ 3. *Records of Stomatal Movement.*

The appliance here described nominally records the velocity with which air escapes through the stomata. The square root of this velocity (when below a certain value) appears to represent more closely the actual stomatal aperture.* Discussion of the difficult physical aspects of the matter is beyond the purpose or the power of the present writer, but attention may be drawn to one systematic error in the appliance.

For low velocities, and until some such upper limit as a "fifteen-second stroke" with the constants of pressure, capacity, and area given above, an increase in the frequency of the stroke is probably directly proportional to increases in mean stomatal aperture; beyond some such upper limit, however, the friction of the out-flowing air along the exit-tube and skin-friction of the liquid on the gas-holder become noticeable, so that when there is no leaf on the chamber the strokes still take about five seconds each, which is the minimum time for leaves with fully-opened stomata. The correction for this would have to be worked out empirically for each appliance.

Neglecting this for the present, the stomatograph can be used for almost any kind of stomatal investigation, either in the laboratory or in the field. Comparison of various leaves on the same plant can be effected rapidly, by substituting one leaf after another on the chamber, as soon as some five strokes of the pump have been recorded. One particular advantage of the original porometer is that its results show the mean condition of many hundreds of stomata in the experimental area; with the stomatograph this advantage can be extended to obtaining the mean of many leaves on the same plant, by employing a number of chambers, all of which are connected to the same pump. Such records might be of interest to students of ecology.

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* Darwin and Pertz, *loc. cit.*

The chief interest attaches, however, to continuous field records, since these are unique, excepting for microscopic examinations made by Lloyd.* Fig. 4 shows the general nature of stomatal behaviour on a single adult leaf of

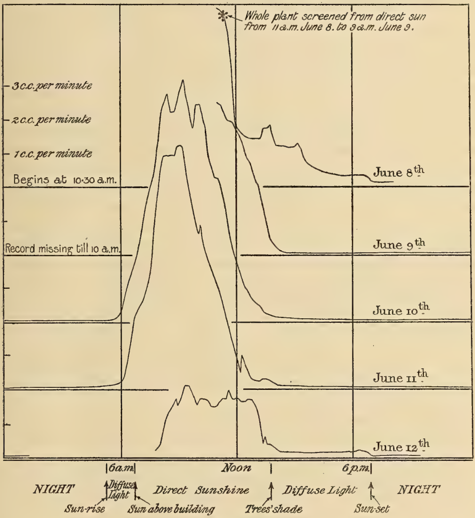

FIG. 4.

Egyptian cotton, growing under field conditions excepting for the presence of the chamber. The behaviour of *Helianthus* in Egypt appears to be closely similar. The curve gives the number of cubic centimetres of air passing through the leaf per minute in each ten-minute period.

The circumstances under which this record was taken were roughly as follows: Sun temperature with black bulb *in vacuo*, 65° C. Maximum shade temperature at 2 P.M., 35° C. Minimum night temperature at 5 A.M., 22° C. Humidity, varying, from 20 per cent. of saturation by day to 100 per cent. by night. The soil had been irrigated seven days before the record begins. Sunshine was direct and continuous, between the limits indicated on the graph, during all five days.

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* *Loc. cit.*

The leaf showed slight signs of injury on the last day, in the portion which touched the wax, but this slight browning had disappeared within a week after its removal from the chamber. The record for the fifth day is plainly abnormal.

The general nature of these and of other similar records is as follows :— At sunrise the stomata open slightly and continue to do so until the direct sun strikes them. They then increase their aperture very rapidly to a maximum at about 9 A.M. After remaining wide open for a longer or shorter time, which appears to depend on the development of the root-system and on the humidity of the air and soil—provided, of course, that sunshine is continuous—they begin to close more and more quickly till their aperture is less than it was before the direct sun reached them. The explanation of this closure seems to be provided by an hypothesis of "water-starvation"; the root-absorption being insufficient to cope with the heavy loss by transpiration, the latter is reduced in consequence.

The closure continues until, on some days, the stomata are practically shut by noon. Preliminary investigations indicate that, in consequence of this, photo-synthesis only takes place during the early part of the day, and that the plant is in a quiescent condition during the afternoon—neither growing* nor feeding, but merely waiting for release from the tyranny of the sun.

In the particular site where these records were taken, the sun passes behind trees at 1.30 P.M., when the stomata close completely. A portion of this effect might be due to the construction of the chamber (3A), and will have to be re-examined next year with the new chamber (3B).

The effect of shading the whole plant from direct sun, though not from bright diffuse light, and so reducing the water loss, is shown in fig. 4 (June 8). The shade was applied before rapid closure had begun, and provoked immediate partial closure, till the aperture was about the same as during pre-sunshine period of the early morning. This aperture was maintained well into the afternoon, and was followed by slow closure until sunset, when the final closure took place almost suddenly.

The stomatograph has thus begun immediately to justify the purpose of its invention by throwing a flood of light on the important problems of water-relationships of the cotton-plant under the very severe conditions of the Egyptian summer. The investigations suggested by considerations of such records as those reproduced here are almost endless, and they have in addition the most direct relation to irrigation matters, which are of great economic importance for Egypt.

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* Balls, W. Lawrence, 'Cairo Sci. Journ.,' September, 1911.

§ 4. *Summary.*

The stomatograph is an air-pump measuring and recording the quantity of air which it forces through a leaf on the chamber of Mr. Francis Darwin's porometer, and so recording any changes in stomatal aperture.

It is extremely easy to use, and having been so constructed as to be independent of weather changes, is specially adapted to obtaining records from plants under normal out-door conditions of environment, such as are required in agricultural and ecological studies.

Records obtained in this way with the Egyptian cotton crop show that the instrument may be of great utility in the study of purely economic matters connected with irrigation.

Apart from its use for the special purpose, the form of pump adopted seems likely to be of use in replacing aspirators for many kinds of scientific research.

My thanks are due to Mr. Francis Darwin for the interest which he has taken in this modification of his porometer, and to Mr. Horace Darwin for suggestions as to its improvement. My colleague, Mr. Frank Hughes, is responsible for many ideas, and for assistance in making the various working models.

DESCRIPTION OF THE FIGURES.

General: The drawings of apparatus are all semi-diagrammatic, sectional and superficial views being combined in the same figure. Figure 4 is plotted from the chronograph records.

FIG. 1A.—Interior of case containing pump, relay, and battery. Gas-holder and solenoid in section, remainder in diagrammatic view. Insulated wire represented by dotted lines, or by spirals. Battery (*CZ*) is actually stored at the back of the case, behind the pump.

p.	Pump nozzle for connection to chamber.
t, t.	Telegraph connections to recording apparatus.
a.	End of rod which depresses the contact arm, *Ca.*
b.	End of similar rod which knocks off the contact arm.
B.	Pillar of balance, bearing quadrant beam in front, and contact arm behind on same pivot.
Ca.	Contact arm, during contact.
Ca′, Ca′.	Normal position of contact arm.
A.	Light armature on contact arm.
M.	Holding down magnet, maintaining contact of *Ca* by means of *A*.
Br.	Bracket supporting insulated fixed contact at tip.

Cr. Contact closing relay circuit. (Dotted line represents position of same when relay circuit is open.)
R. Relay magnet.
Ex. Rubber exit tube from gas-holder to nozzle *p*, passing through a condensed-water trap-bulb.
G. Gasholder, with—
V, V. Inlet valve holes.
L. Liquid (paraffin, water, or mercury) in which gas-holder floats.
Bp. Baffle plate at foot of gas-holder, carrying ring-weights.
C. Soft iron core.
S. Solenoid.

Fig. 1B.—Plan of upper portion of pump to show arrangement of contact arm. Lettering as in Fig. 1A.

Fig. 2.—Semi-sectional view of chronograph. Drum represented by dotted lines.
P. Electro-magnetic marker, with pen.
r. Glass rod, bearing brass spiral.
sp. Brass wire spiral.
g, g. Driving rods, rotated by " L " pieces on hour spindle of clock.
Cl. Clock.
c. Upper detachable end of drum cylinder.
n. Lower ditto, with three-point nut.

Fig. 3.—New pattern of porometer chamber, connected in use to nozzle *p* of pump.

3A. Chamber made from cork and glass, with grooved flange containing paraffin wax, as used in obtaining fig. 4.

3B. Improved chamber, also with the waxed flange, but transparent at back.

Fig. 4.—Record for five consecutive days, converted to a curve, showing changes in stomatal aperture as indicated by the variation in volume of air under constant pressure which can be forced through the leaf-tissue. Plotted in terms of c.c. per minute, through an area of leaf of 80 sq. mm., under a pressure of 0·5 mm. of mercury. Classified in 10-minute intervals.

Excepting on the first and fifth days the results represent the normal behaviour of the Egyptian cotton plant (*Gossypium peruvianum?*) under conditions which are nearly identical with those of the field crop, excepting for some shading from direct sun in the early morning and after 2.30 P.M., on account of adjacent trees and buildings.

The leaf employed had just reached its full dimensions, on a plant with 10 leaves, growing in the garden attached to the laboratory of the Khedivial Agricultural Society, temporarily occupied by the Egyptian Government Department of Agriculture.

Further Experiments on the Cross-breeding of Two Races of the Moth Acidalia virgularia.

By W. B. Alexander, B.A., late Vintner Exhibitioner of King's College, Cambridge.

(Communicated by Prof. E. B. Poulton, F.R.S. Received January 3,—Read February 22, 1912.)

A communication on the heredity of the two forms of this species was read to the Royal Society on February 25, 1909. In that paper Messrs. Prout and Bacot gave an account of a large number of broods of this moth reared by them for nine generations.

At the end of the paper they mentioned that they had handed to Mr. W. Bateson ova produced by pairings in generation F_{10}. It was the larvæ derived from these ova which Mr. Bateson gave into my charge in February, 1909.

Of the five broods which I thus obtained I only managed to continue two, as the individuals of the others emerged at such long intervals that I never had a male and female alive at the same time. Fortunately, however, the two I reared were the most interesting.

Brood 2.—This brood had been labelled DI✻, gen. xi, by Mr. Bacot. Eleven moths emerged (4 ♂, 7 ♀) and from them I obtained two lots of ova—Broods 6 and 7. From Brood 6 I reared 35 moths (16 ♂, 19 ♀) and obtained three more lots of ova—Broods 9, 11, and 12. Brood 11 was liberated in the larval stage; from Brood 9 I reared 64 moths and from Brood 12, 38 moths (23 ♂, 15 ♀). From Brood 7 I reared 30 moths (14 ♂, 16 ♀) and obtained one lot of ova—Brood 13; from which I reared 92 moths (45 ♂, 47 ♀).

Thus of this strain I reared no less than 270 individuals, distributed in six families and three generations, and the striking feature was that they showed no appreciable variation. They were all of a yellowish colour with a slight amount of dark speckling on the wings.

I had not at this time seen any specimens of the original light form of the species, var. *canteneraria,* from Hyères, but when, through the kindness of Prof. Poulton, I was enabled to examine Messrs. Prout and Bacot's specimens in the Hope Collection at Oxford, I saw at once that all the descendants of my Brood 2 should certainly be classed as *canteneraria.* They did not show any more speckling of black than typical forms of that variety, but their yellow ground-colour was much darker than that of typical *canteneraria,* though certain males, even reared from Hyères eggs direct, were as yellow as my moths.

The history of this race appears to be as follows:—Some light forms appeared among the dark ones in generation 6 in a box which was supposed to contain the pure dark strain. A number of the descendants of this brood were reared and in general gave a mixture of pure light forms and forms intermediate between light and dark. In some cases where two of these light individuals were mated they yielded nothing but lights, in others a mixture of lights and intermediates. On the other hand the darkest forms continued to throw light individuals when mated together. The actual parents of my Brood 2 appear not to be known, but there is no doubt that they were members of the generation 4 in descent from the original aberrant individuals. We thus see that it took five generations to establish a pure light brood from the original light individuals whose origin was quite inexplicable.

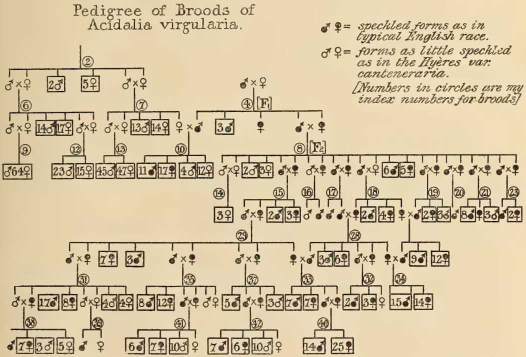
Brood 4.—This was the brood labelled DxLMxi by Mr. Bacot. They were the result of mating a pure dark male with a pure light female, both of whose ancestors had been reared in captivity for 11 generations. It was thus what is ordinarily known as an F_1 brood, though readers of Messrs. Prout and Bacot's paper will note that, though they claim to have numbered their broods according to "the well-known Bateson method," they have not confined F_1, F_2, etc., to the first, second, etc., generations of a hybrid strain, but have given these numbers also to broods of the pure strain, dating arbitrarily from the broods which were first reared in confinement.

My F_1 brood consisted of seven moths (5 ♂, 2 ♀), all of them distinctly speckled with black to a greater extent than in the light form, whilst their ground colour was a dirty white, not nearly so yellow as in my Brood 2.

From this brood I obtained one lot of ova—Brood 8, both of whose parents were of the type just described, and another lot of ova from a male of this brood mated with a female of Brood 7 (one of the pure yellow *canteneraria* broods descended from Brood 4). This I labelled Brood 10.

Brood 10.—This brood, consisting of 44 moths, possessed the yellow ground colour of its mother throughout, but 28 of the moths were distinctly speckled like their father, whilst 16 were exactly like their mother. This points to the ratio of 2 speckled : 1 unspeckled, but I think the numbers in this brood must be distrusted, since for some reason there were also 29 females and 15 males, which again suggests a ratio of 2 to 1, though of the total number of moths I have examined 305 were males and 309 females.

Brood 8.—This was the F_2 brood of 34 individuals, and exhibited a very considerable amount of variation, much more than that in F_1. I found, however, that it was quite easy to distinguish 9 moths as having no more speckling than pure *canteneraria*, whilst the remaining 25 were distinctly more speckled. Of the nine unspeckled (4 ♂, 5 ♀), three males were as yellow as the individuals of Brood 4, one distinctly paler, the females were all paler, two of them as white as the lightest of the pure Hyères race. It thus appeared that about one quarter (9 out of 34) of the F_2 brood were referable to the pure light race, though several of the males were yellower than any but extreme examples of it. The numbers suggested at once that the unspeckled Hyères race was recessive to the speckled English form. I reared a number of further broods from pairs of the F_2 moths, and with one exception (Brood 39) this supposition would account for the composition of all the succeeding broods (see Pedigree).



Pedigree of Broods of Acidalia virgularia.

♂ ♀ = *speckled forms as in typical English race.*

♂ ♀ = *forms as little speckled as in the Hyères var. canteneraria.*

[Numbers in circles are my index numbers for broods]

I do not think it is necessary to deal with the subsequent broods in so much detail, as their mutual relationships will be visible from the pedigree.

The last generation emerged at irregular intervals in the early months of 1911, and only 3 females laid eggs. From one set of ova a few larvæ emerged, but only lived for a few days. Thus the race became extinct, perhaps owing to complete inbreeding for six generations.

The descendants of Brood 8 may be classified as follows :—

Both Parents Unspeckled (Broods 14 and 39).—Offspring, 4 unspeckled, 1 speckled. The one speckled moth resulting from this type of mating in Brood 39 is the only serious difficulty in the way of accepting a normal Mendelian relationship between speckled and unspeckled types. If it stood entirely alone I should have to regard it as due to some sort of error, though I did my utmost to guard against mistakes. But in the history of Messrs. Prout and Bacot's aberrant strain already mentioned, and in some of their other broods, there are definite cases of speckled forms originating from two non-speckled parents. Possibly one of the parents in these cases is a heterozygote indistinguishable in appearance from a recessive.

Both Parents Speckled.—In some broods (15, 17, 19, 20, 23, 29, 28, 30, 33, 34, and 40) all the offspring are speckled; in others (8, 21, 31, 35, 37, 32, 41, and 42) some of the offspring are non-speckled.

These eight broods consist of 161 moths, of which in theory $\frac{1}{4}$, or 40, should be non-speckled, and 121 speckled. Actually we find that 51 were non-speckled and 110 speckled.

One Parent Speckled and One Parent Non-Speckled.—In one brood (18) all the offspring were speckled; in the remainder (10, 16, and 38) some of the offspring are non-speckled. These three broods consist of 62 individuals, of which theoretically 31 should be speckled and 31 unspeckled. Actually we find that 37 were speckled and 25 non-speckled.

These numbers are sufficiently near those demanded by theory to give considerable support to the theory that the factor which causes the wings of the English form to be much speckled with black is dominant to its absence in var. *canteneraria*. A study of the much larger numbers reared by Messrs. Prout and Bacot, now in the Oxford Museum, reveals the fact that though this is evidently an approximation to the truth, it is not universally true. For in several of their F_1 generations a few non-speckled moths occur, the numbers being as follows:—

A	58	speckled,	2	non-speckled
C	3	„	0	„
c	7	„	0	„
h	12	„	0	„
H	56	„	0	„
G	68	„	1	„
*g**	20	„	3	„
	—		—	
7 F_1 broods,	224	„	6	„

* Several much rubbed not included.

These figures again suggest that heterozygotes may occasionally be indistinguishable from recessives. This, as already mentioned, would account for all the anomalies met with so far.

I have studied also the F_2 families at Oxford, grouping them also into speckled and non-speckled. I find that they are:—

A	65 speckled,	0 non-speckled.	
E	32 „	12 „	
e	38 „	10 „	
C	42 „	12 „	+several much rubbed.
c	27 „	3 „	+a few „
h	22 „	12 „	+ „ „
H	33 „	11 „	+ „ „
F	94 „	56 „	a few doubtful.
g	2 „	2 „	
G	4 „	0 „	doubtful, a good deal rubbed.
10 F_2 broods,	359 „	118 „	

These numbers appear very good, as theoretically we should expect 358 : 119, but it will be noticed that Broods A and F compensate for one another. Brood A is definitely exceptional; Brood F is a composite one derived from a number of females; this should, of course, make no difference to the numbers, but if separated it might have been found that one female produced only light offspring as A produced only dark ones.

Taking away A and F we have 200 speckled to 62 non-speckled, where expectation would be 197 speckled to 65 non-speckled—a very close agreement.

It would thus appear that speckling is an ordinary Mendelian dominant to the absence of speckling, but that whilst in most cases the heterozygous individuals resemble the dominant they may occasionally be indistinguishable from the recessive. Perhaps it would be more accurate to say that speckling is usually dominant to non-speckling, but that occasionally non-speckling is dominant to speckling.

The speckled individuals vary from moths whose wings are only slightly more speckled than in var. *canteneraria* to moths whose other markings are almost obscured by black scales. I believe that this variation is met with among the specimens taken wild in England, but the dark ancestors of my moths were of the most thickly speckled type found in the neighbourhood of London. This type occurred at intervals among the descendants of my Brood 4, and I think it is probably the homozygous speckled type, especially as Broods 33 and 40, consisting of 16 and 39 individuals respectively, maintained this type with practically no variation for two generations. It is impossible, however, to draw a line between this type and less speckled forms, as in some broods there is a perfect gradation down to the (so-called) non-speckled type.

The original London moths differed from any of my rearing in being suffused with a brown colour, which was, however, approached by some three of the most speckled individuals in my Brood 8, which were all males. Among the Oxford specimens there are some of this brown colour without speckling, so that it is evidently due to a factor independent of the speckling. I cannot account for the lack of inheritance of this colour nor for the various yellow and whitish ground-colours among my own moths. As already mentioned, the descendants of Brood 2 all had a uniform yellow ground-colour. Amongst the unspeckled descendants of Brood 4 the males were mostly of this same yellow colour and the females invariably lighter; the same is true of the pure *canteneraria* from Hyères, in which, however, only occasionally are the males yellow like my Brood 2, the majority being much paler. I have found it impossible to classify the speckled moths according to ground-colour, as, when they are much speckled, this is difficult to estimate. In general, however, the females are lighter than the males.

There is another respect in which these moths vary to a considerable extent, and that is in size. The following table shows the numbers of moths among the descendants of Brood 4, of various breadths across the wings:—

	Millimetres.										
Numbers of	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.
Males…………	—	1	6	29	57	66	36	8	—	—	—
Females………	—	—	—	5	11	24	49	66	35	12	—
Totals ……	—	1	6	34	68	90	85	74	35	12	—

It will be seen that these figures form a normal curve, which is due to the combination of two curves, one of males with an average of about 17·5 mm., and one of females with an average of about 19·5 mm.

Now, some of the individual broods depart markedly from these averages, *e.g.* :—

Numbers of individuals of	Millimetres.											Average. mm.
	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	
Brood 40 ♂…	—	—	—	—	1	4	7	2	—	—	—	18·4
♀…	—	—	—	—	—	—	1	7	12	4	—	20·7
Brood 34 ♂…	—	1	3	7	4	—	—	—	—	—	—	15·9
♀…	—	—	—	2	8	2	1	—	—	—	—	17·2

It would be natural to suppose that such a great difference in size between two broods would be due to heredity or to some great difference in the conditions in the larval stage, but I can find nothing of the sort to account for it.

The parents of Brood 40 measured ♂ 18·0, ♀ 20·3, whilst those of Brood 34 measured ♂ 17·9, ♀ 19·6.

The parents of 40 were both members of 33, which averaged ♂'s 17·7, ♀'s 19·7; the ♂ parent of 34 belonged to 30, which averaged ♂'s 17·0, ♀'s 18·7, whilst the ♀ parent of 34 belonged to 28, which averaged ♂'s 17·5, ♀'s 18·2. It is true that the ancestors of Brood 34 were distinctly smaller than those of Brood 40, but they did not differ from the normal nearly so widely as does Brood 34 itself; and, taking into account all broods containing over 10 moths, I find that the average spread of the brood shows no correlation with that of its parent nor with those of the broods of which the parents were members. I conclude, therefore, that breadth of wing is not hereditary.

It is, of course, impossible to prove that these variations in size are not due to differences of conditions, though most of the conditions have been kept very uniform. The moths were reared throughout in similar boxes and the larvæ never fed on anything but dandelions. The main difference of conditions between the broods was due to the different temperatures of different periods of the year. This has a marked effect on the length of time required for development. Eggs laid from April to August only take about two months to reach the imago stage, whilst eggs laid from September to November take about four months if the larvæ continue feeding, or from seven to eight months if they definitely hibernate and cease feeding.

In spite of these great differences, moths of the broods which have hibernated are, on the average, exactly the same size as those which have completed their development in two months.

If the differences were due to different temperatures at the moment when the moths emerged, those broods which emerged from July to October must have experienced warmer conditions for emergence than those which emerged from December to June, yet on the average there is no difference between the two groups. I am therefore entirely at a loss to explain the considerable variations in size which occur.

Summary.

It will be seen that I agree with Messrs. Prout and Bacot's conclusion that *Acidalia virgularia* and its variety *canteneraria* are not two Mendelian forms of the species, though I believe I have shown that one of the differences between the two forms, namely, the speckling of *virgularia,* behaves in most cases as a Mendelian dominant to the absence of this speckling in *canteneraria.* Even to this rule I am bound to admit there are certain exceptions. To account for this I have to assume that, whilst the heterozygote is usually more or less like *virgularia,* it may sometimes be indistinguishable from *canteneraria.*

The other difference between the species and its variety is that *virgularia* is often, and in the London form always, much browner or yellower in ground-colour than *canteneraria.* Of the various colours of ground seen in the hybrid race I have been able to suggest no explanation, though, as already noted, a certain amount of order is discernible, especially in the fact that the males are almost invariably darker than the females.

In regard to size it has been shown that, taken as a whole, the individuals fall into a normal curve of error, but that some of the broods show considerable departures from the normal, for which no explanation in heredity or environment is forthcoming.

It should be mentioned also that both speckled and non-speckled, yellow and white individuals occurred in broods which hibernated as larvæ as well as in broods which passed through their whole development in a couple of months in the summer, so that these differences are not likely to be due to environment, nor are they seasonal forms.

The specimens have been placed, with Messrs. Prout and Bacot's material, in the Hope Department of the Oxford Museum.

An Instrument for Measuring the Distance between the Centres of Rotation of the Two Eyes.

By H. S. Ryland and B. T. Lang, F.R.C.S.

(Communicated by George J. Burch, M.A., D.Sc., F.R.S. Received January 9,—Read February 29, 1912.)

A knowledge of the distance between the two eyes being required in dealing with the problems of binocular vision, the instruments described in this paper may be of interest to physiologists. All those methods which involve measurement of the distance between the pupils (or other external parts of the eyes) are liable to errors, for the following reasons:—

1. It is difficult to eliminate parallax between the scale or index of the measuring instrument and the eyes.

2. The distance between the pupils is affected by the convergence of the optical axes, and still more by the abnormal direction of either.

3. It is difficult to ensure the eyes being kept still during the process of measuring.

By the method herein described, advantage is taken of the mobility of the eyes, which is the cause of this very difficulty, to measure the one distance that cannot vary, namely, that between the optical centres of rotation. So far as we have been able to find, this has not been done before. We have made two instruments, each involving the same general principles in a different form.

The first form (fig. 1) consists of a suitable base A, shaped at one end to fit the forehead, and carrying at the other end a vulcanite plate B, upon which slides a vertical index C, against a scale SS. In the base itself there is a vertical index which may be placed at either D or E. When in use

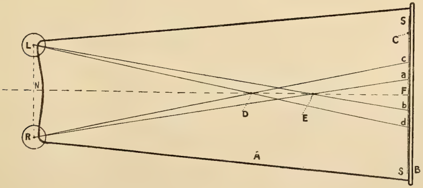


Fig. 1.

the instrument is held in front of the face with the shaped end against the forehead. One eye, say the left, is covered, and the patient is instructed, the first index being at D, to move the scale index along the plate B until it apparently lies just behind the first index at a. Without altering the position of the instrument, a similar observation is made with the other eye, the scale index being moved to b. The first index is now moved to E, and similar observations taken for each eye, giving the readings c and d respectively for the scale index.

Then (in fig. 1) join LR, and produce DE to F and to N. Let the distance $ab = y$, $cd = y'$, $\mathrm{EF} = x$, $\mathrm{ED} = d$, $\mathrm{ND} = z$.

Let R be the position of the right eye and L that of the left, and let $\mathrm{RL} = \mathrm{V}$ be the distance between the centres of rotation. Then V may be determined graphically by a simple construction, or, assuming the base line RL to be parallel to SS, may be calculated as follows :—

From fig. 1 we have

$$\frac{\mathrm{V}(d+x)}{y'} = z \quad \text{and} \quad \frac{\mathrm{V}x}{y} = z+d,$$

from which

$$\mathrm{V}\left(\frac{x}{y} - \frac{d+x}{y'}\right) = d, \text{ or } \mathrm{V} = \frac{d\,(yy')}{xy' - y\,(d+x)}.$$

In practice, as only two variables occur in this equation, namely y and y', a table may be computed and used with the instrument.

In the second form (fig. 2) the instrument has three fixed vertical indices, K, L′, and M. The vulcanite plate and sliding index are similar to those employed in the first form. With the left eye covered, the white index is moved till it is apparently behind L′ at p, and M at n. Similar observations are taken with the left eye, the scale index being moved till it is apparently behind the indices K and L′ at q and s respectively. As with the first instrument described, the distance $\mathrm{RL} = \mathrm{V}$ may be obtained from a simple

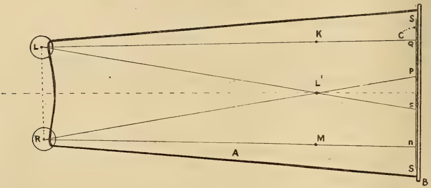


Fig. 2.

geometrical construction, or, assuming parallelism between the base line RL and the scale SS, V may be calculated from the following data :—

Let the distance KM $= a$, $ps = b$, $qn = c$, RL $=$ V.

Then from fig. 2 we have

$$b : \mathrm{V} = c-a : a-\mathrm{V}, \quad \text{or} \quad ab-b\mathrm{V} = (c-a)\,\mathrm{V};$$

that is

$$\mathrm{V} = ab/(b+c-a).$$

Since a is constant for a given instrument, the values of V may in practice be obtained from a table computed in terms of b and c.

Further, it may be pointed out that in all the methods hitherto employed for measuring the distance between the centres, it is essential that the subject should have binocular vision, in order to enable him to fix an object with both eyes simultaneously. By either of the methods just described it is possible to measure the distance between the centres of persons who cannot fuse the images of the two eyes, or even, provided the vision of each eye be sufficient, to measure this distance in the case of persons who squint. Moreover, it is not necessary that the vision should be distinct so long as the eye can distinguish the index. Good measurements can be made even though the definition is not sufficient to enable large print to be read. Various methods may be used to increase accuracy of scale reading, such as verniers and multiplying levers.

Composition of the Blood Gases during the Respiration of Oxygen.

By George A. Buckmaster and J. A. Gardner.

(Communicated by Dr. A. D. Waller, F.R.S. Received January 9,—Read February 22, 1912.)

(From the Physiological Laboratory, University of London, South Kensington.)

The effect of breathing oxygen-rich gas mixtures on men and animals has been frequently investigated. The papers which we have consulted deal exclusively with methods in which the respiratory exchange was studied, and the general results of the more recent experiments confirm the view that there is little or no difference in metabolism, as indicated by the gaseous exchange, whether ordinary air or gas mixtures rich in oxygen are respired.* A contrary opinion has been formed by some observers, such as Rosenthal† and Lukjanow.‡ We have been unable to find any paper later than the work of P. Bert which deals with the composition of the blood gases during oxygen inhalation. While engaged on a study of the nitrogen-content of the blood we accumulated a mass of data on the comparative quantities of carbon dioxide and oxygen in the blood of cats breathing air and high percentages of oxygen. These results we bring forward in this paper.

The cats were anæsthetised with urethane. The process for obtaining the samples of blood, the precautions to be taken in the evacuation of the gases by the tapless form of blood pump, were those described at length in a former paper,§ in which the mode of administration of oxygen has also been fully described. Respiratory tracings were taken in every case, but we do not consider it necessary to reproduce these.

In many of the experiments, but unfortunately not in all, as these were originally made with another object, determinations were made of the hæmoglobin content by the Gowers-Haldane hæmoglobinometer.

It has been stated by August Krogh that "the hæmoglobin of different animals does not necessarily possess the same relative affinity for oxygen and carbonic oxide."|| If this is true, then the hæmoglobin of the blood of

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* (1) Speck, 'Archiv f. d. gesammte Physiologie,' 1879, vol. 19, p. 171; (2) Loewy, 'Übersuchungen über die Respiration und Circulation,' Berlin, 1895; (3) Arnold Durig, 'Archiv f. Physiologie,' 1903, Supp. Bd., p. 209; (4) Schaternikoff, 'Archiv f. Physiologie,' 1904, Supp. Bd., p. 135; (5) F. G. Benedict and H. L. Higgins, 'American Journal of Physiology,' 1911, vol. 38, p. 1.

† Rosenthal, 'Archiv f. Physiologie,' 1902, Supp. Bd., p. 293.

‡ Lukjanow, 'Zeits. f. physiol. Chemie,' 1884, Bd. 8, p. 324.

§ "The Nitrogen Content of the Blood," 'Journ. Physiol.,' 1912, vol. 43, No. 6, p. 401.

|| 'Skand. Arch. Physiol.,' 1910, vol. 23, p. 220.

different animals cannot be identical in all respects. C. G. Douglas,* using rabbits as the subject of his experiments and the Gowers-Haldane hæmoglobinometer to determine the percentage oxygen capacity of blood, considers that a parallel relationship exists between the oxygen capacity of the blood and the depth of its colour as judged by the hæmoglobinometer, and it was essential for our purpose to ascertain if this was true for cats.

In the case of cats we have found that there is complete agreement between the actual values for oxygen as determined with our pump and that of the oxygen capacity by the hæmoglobinometer. We used hirudinised blood of cats; the saturation method, determined *in vitro* at 38° C., has been described in a former paper.†

Percentage Oxygen Capacity of Blood.

	By pump.	By hæmoglobinometer.
A	16·52	17·39 hirudin.
B	21·04	19·89 defibrinated blood.
C	19·48	19·89 hirudin.
D	17·24	16·56 ,,
Mean	18·57	18·43

Difference +0·14 c.c. per 100 c.c. blood.

This supports the contention of those who believe that no valid argument exists against the view that the hæmochromogen moiety of the molecule of hæmoglobin is identical in all animals.

Composition of the Blood Gas for Animals Breathing Air and Oxygen respectively.

In Table I we give for cats under urethane anæsthesia :—

1. The composition of the blood gases.
2. Percentage hæmoglobin value.
3. Calculated theoretical oxygen capacity per 100 grm. of blood.
4. Calculated percentage saturation of hæmoglobin.
5. Calculated percentage saturation of hæmoglobin from the percentage composition of alveolar air, making use of the blood dissociation curve given by J. Barcroft and M. Camis for dog's blood.‡
6. The percentage composition of alveolar air.

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* 'Journ. Physiol.,' 1910, vol. 39, p. 453.

† 'Journ. Physiol.,' 1912, vol. 43, No. 6, p. 410. In Experiment XIV, Table III, the second oxygen figure in the blood-gas analysis should read 17·24 instead of13·59.

‡ 'Journ. Physiol.,' 1909, vol. 39, p. 132.

Table I.—Arterial Blood (Carotid Artery) of Cats Breathing Air.

No. of experiment.	Weight of cat.	Hirudin.	Volume of blood.	Blood gas per 100 c.c. blood.				Percentage of hæmoglobin.	Theoretical oxygen capacity.	Calculated percentage saturation of hæmoglobin.	Calculated percentage saturation of hæmoglobin.*	Alveolar air.			Remarks.
				Total.	CO_2.	O_2.	N.					CO_2.	O.	N.	
	kgrm.		c.c.	c.c.	c.c.	c.c.	c.c.								
I	3·2	+	21·5	50·07	34·52	14·50	1·05								
II	2·7	+	21·44	47·91	33·68	13·10	1·14								
III	2·8	+	10·3	53·80	37·48	15·22	1·12	84	15·54	97·4					
IV	2·5	+	10·3	51·57	35·76	14·89	0·91	84	15·54	95·8	—	5·0	—	—	Respiration frequency 43 per min.
VI	2·7	+	10·3	50·15	33·65	15·29	1·22	92	17·02	89·8	88·0	3·95	13·56	82·47	72 per min. resp. rate.
VII	3·7	+	10·3	50·03	33·09	15·96	0·97	95	17·58	90·8	90·5	2·68	15·23	82·09	Resp. rapid; average 73 per min. just before taking sample.
VIII	2·7	+	10·3	64·71	47 84	15·66	1·22	103	19·15	81·7	86·0	6·24	9·15	84 64	Blood rather dusky, but tracings before and after sample showed lung ventilation not very efficient in depth and frequency; rate 15—16 per min.
IX	1·9	+	10·3	56·26	43·58	11·58	1·09	92	17·02	68	87·2	6·38	11·61	82·01	
X	2·5	—	10·3	51·17	38·08	12·03	1·07	80·5	14·89	80·8	87·0	6·25	9·74	84·04	
XI	—	—	10·3	57·17	38·68	17·45	1·07	105	19·42	89·7	87·5	5·74	9·66	84·59	Shallow, panting respiration.
XII	2·6	+	10·3	60·70	49·48	10·01	1·21	96	17·76	86·3	—	—	—	—	Blood dark. Sample taken 4 hours after urethane.
XIII	2·6	+	10·3	55·54	42·03	12·56	0·95	95	17·57	71·5	—	—	—	—	Same cat as XII, 1 hour later.
XIII *a*	3·3	+	10·3	50·01	31·77	16·68	1·55								
Mean values ……				53·76	38·43	14·22	—	—	17·15	83	—	—	—	—	

* From curve of dissociation given by Barcroft and Camis.

Table II.—Arterial Blood (Carotid Artery) of Cats Breathing Oxygen.

No. of experiment.	Weight of cat.	Hirudin.	Volume of blood.	Blood gas per 100 c.c. blood.				Hæmoglobin, Gowers-Haldane.	Theoretical oxygen capacity.	Calculated percentage saturation of hæmoglobin.	Alveolar air.			Duration of oxygen.	Remarks.
				Total.	CO_2.	O_2.	N.				CO_2.	O.	N.		
	kgrm.		c.c.	c.c.	c.c.	c.c.	c.c.							mins.	
XVIII	2·2	+	21·5	49·62	38·38	10·78	0·48	72	13·32	80·93	5·80	89·48	4·71	78	
XIX	2·6	+	21·45	67·42	50·08	17·16	0·18	—	—	—	7·58	86·76	5·95	89	
XX	2·2	+	21·5	51·56	40·79	10·60	0·18	—	—	—	19·43	79·86	0·73	77	Respiration shallow and slow.
XXI	3·6	+	21·5	50·77	33·56	17·07	0·12	81·5	15·08	113·4	3·84	94·33	1·81	78	Normal respiration.
XXII	3·0	+	21·5	60·26	41·59	18·28	0·39	85·5	15·82	115·5	8·31	88·84	2·84	84	Normal respiration.
XXIII	2·8	+	10·3	47·71	32·83	14·82	0·06	83	15·36	96·5	—	—	—	72	Irregular and shallow.
XXIV	2·5	+	10·3	47·01	33·09	13·85	0·08	84	15·54	89·15	—	—	—	55	Rapid and somewhat deep respiration.
XXV	2·7	+	10·3	54·98	38·28	16·62	0·07	89	16·46	101·6	4·65	92·77	2·58	48	Rapid and deep.
XXVI	3·7	+	10·3	39·37	26·90	12·30	0·17	94	17·39	70·6	5·00	92·98	2·01	35	Rapid; animal disturbed by retching movements before sample.
XXVII	2·7	+	10·3	64·54	45·65	18·81	0·08	103	19·15	98·24	10·97	86·03	3·00	95	Slow and shallow.
XXVIII	3·3	+	10·3	54·06	37·99	15·99	0·07	—	—	—	—	—	—	77	Normal respiration.
XXIX	2·8	+	21·55	50·84	40·00	10·74	0·10	104·5	19·33	55·50	—	—	—	110	
XXX	3·1	+	21·5	61·01	43·18	17·21	0·12	104	19·24	89·45	8·20	88·88	2·95	108	Ventilation of lung exceedingly good. 1·50 per m., resp. very deep, 28 per m. 1·55 ,, ,, 40 ,, 2·25 ,, respiration shallower.
Mean values ……				53·79	38·65	14·94	—	—	16·67	91·09*					

* Or, by calculation from mean values, 89·62.

In Table II we give similar data with the respiration of oxygen, the samples of blood for analysis and those of alveolar air being taken during the period of oxygen inhalation; the alveolar air *immediately* after the blood sample.

On comparing these two tables, it will be seen that the average blood gases in the two series of experiments are in close accord, indeed practically identical.

Average of Thirteen Experiments on Cats breathing Air and Oxygen.

	Air.	Oxygen.
Total gas	53·76	53·79
Carbon dioxide	38·43	38·65
Oxygen	14·22	14·94

In some experiments blood was abstracted from the same animal when breathing first air and subsequently oxygen.

A contrast of these duplicate samples of arterial blood of animals breathing air and oxygen is given below:—

Air.		Oxygen.	
CO_2.	O_2.	CO_2.	O_2.
47·84	15·66	45·65	18·81
33·09	15·96	26·90	12·30
33·65	15·29	38·28	16·62
35·76	14·89	33·09	13·85
37·48	15·22	32·83	14·82
31·77	16·68	37·99	16·99

It will be seen that, although the percentage of hæmoglobin is practically the same in the same pair of experiments, the oxygen in the blood is sometimes a little greater, sometimes less, when oxygen is inhaled, differences which appear to be independent of the percentage of oxygen in the alveolar air.

The hæmoglobin values in the several experiments are very variable in cats. The theoretical oxygen capacity of cats breathing air, ascertained from 10 experiments in which the hæmoglobin values were determined, was 17·15 c.c. per 100 c.c. of blood. Calculating from the average oxygen found, 14·22, the average percentage saturation of hæmoglobin is about 83.

In the case of animals breathing oxygen, the theoretical oxygen capacity calculated from 10 experiments is 16·67, giving an average percentage saturation of the hæmoglobin of 89·62; 6·7 per cent. higher than in the case of animals breathing air. But it is noticeable that the average hæmoglobin percentage for cats breathing air is 92·65, and for 10 cats respiring oxygen it is only 90·05. It would seem evident from these experiments that the inhalation of oxygen does not necessarily augment the quantity of oxygen in the blood, nor does it appear to affect the quantity of carbon dioxide.

On reference to Table II it will be seen that the average carbon dioxide-content of arterial blood in 13 experiments was 38·43 c.c. per 100 c.c. of blood, or 70·9 per cent. of the total gas. With animals in a state of hyperpnœa,* this falls to an average of 20·56 c.c. per 100 c.c. of blood, or 58·7 per cent. of the total gas, the oxygen remaining practically the same. In experiments with hirudinised blood *in vitro* the carbon dioxide is only 3·83 c.c. per 100 c.c. of blood, or 16·3 per cent. of the total gas.

	Cats breathing air (average of 13 experiments.	(1) Cats in state of hyperpnœa (average of four experiments).*	(2) Blood shaken in air until saturated with oxygen (average of four experiments).†
Total gas	53·76	35·02	23·45
CO_2	38·43	20·56	3·83
O_2	14·22	13·50	18·57

The oxygen-content is of some importance. In Table II the average oxygen-content per 100 grm. of blood in 14 experiments is 14·22 c.c. In the various experiments the values vary considerably, from 10 c.c. to 17 c.c., and the percentage saturation of hæmoglobin is also variable, the average being 83 to 84 per cent. In the cats in a state of hyperpnœa the average is 13·5 not very different from many of the values found in quiet respiration. It would appear from our experiments that the state of hyperpnœa affects the carbon dioxide output rather than the oxygen intake. From the experiments we have already published, it is clear that it takes a considerable time for blood *in vitro* to become saturated with oxygen at 38° C., when exposed to air.

From these experiments it is a fair conclusion that during its passage through the pulmonary capillaries the blood is rarely fully saturated with oxygen even when oxygen is inhaled. For an explanation, it is probable that parts of the lung, for example the apices, are imperfectly ventilated, and also, since the circulation time in the lung is only about five or six seconds, that complete equilibrium is not attained between the blood and alveolar air.

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* 'Journ. Physiol.,' 1910, vol. 41, p. 61.

† 'Journ. Physiol.,' 1912, vol. 43, No. 6, Table III, p. 410.

In the case of venous blood, we have only made one experiment in a cat breathing air.

Venous Blood.

Weight of cat.	Hirudin.	Volume of blood.	Total gas.	CO_2.	O_2.	N.
3·7	+	c.c. 21·5	56·66	44·24	11·31	1·12

We have, however, made a number of determinations of the gases of human venous blood. This was withdrawn from the median basilic vein by displacement of mercury in the manner described in detail in a former paper.* As we performed the experiments on ourselves, the blood was not hirudinised and was taken without an anæsthetic. The results are given in Table III.

Table III.—Venous Blood (Human). Air.

No. of experiment.	Source of blood.	Sample of blood.	Gas per 100 grm. blood.				Remarks.
			Total.	CO_2.	O_2.	N.	
		c.c.	c.c.	c.c.	c.c.	c.c.	
A	J. A. G.	21·37	55·59	44·05	10·27	1·27	No bandage round forearm. Early form of new pump.
B	G. A. B.	18·7	61·46	50·95	9·02	1·47	No bandage on forearm. Early form of new pump.
C	J. A. G.	21·5	54·58	49·07	3·39	2·11	" " "
D	G. A. B.	21·5	59·94	53·33	4·80	1·82	" " "
E	J. A. G.	21·4	55·04	47·10	5·56	2·40	" " "
F	"	21·45	53·39	41·14	10·80	1·47	" " "
G	G. A. B.	21·1	63·22	52·47	9·10	1·65	Taken without any straining of muscles, but after some exercise. Early form of new pump.
H	"	16·5	57·89	48·44	7·79	1·64	Early form of new pump.
I	"	19·0	58·56	46·31	10·21	2·05	" " "
J	J. A. G.	10·3	57·17	50·73	5·37	1·07	Bandage on forearm, not uncomfortably tight. New pump; vacuum with liquid air.
K	"	10·3	57·19	53·63	2·63	0·96	Bandage on forearm, a little tighter. New pump; vacuum with liquid air.

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* 'Journ. Physiol.,' 1910, vol. 40, p. 373.

Table IV.—Venous Blood of Cats Breathing Oxygen.

No. of experiment.	Weight of cat.	Hirudin.	Volume of blood.	Blood gas per 100 c.c. blood.				Hæmoglobin.	Calculated percentage saturation of hæmoglobin.	Theoretical oxygen capacity.	Alveolar air.			Source of blood.	Duration of oxygen.	Remarks.
				Total.	CO_2.	O_2.	N.				CO_2.	O_2.	N.			
	kgrm.		c.c.	c.c.	c.c.	c.c.	c.c.								mins.	
XXXI	3 ·2	+	21 ·2	39 ·77	35 ·93	3 ·59	0 ·26	—	—	—	—	—	—	Right heart ...	80	
XXXII	3 ·0	+	20 ·8	49 ·90	43 ·90	5 ·71	0 ·28	—	—	—	—	—	—	Inferior vena cava	120	
XXXIII	3 ·5	+	21 ·45	58 ·99	51 ·24	7 ·39	0 ·36	—	—	—	7 ·21	88 ·00	4 ·8	Inferior vena cava	95	Cannula slipped for a moment from trachea 15 mins. before sample of blood taken.
XXXIV	2 ·2	+	21 ·45	67 ·70	53 ·89	13 ·49	0 ·33	—	—	—	10 ·71	87 ·04	2 ·25	Right jugular into heart	55	
XXXV	2 ·4	—	21 ·5	61 ·86	51 ·39	10 ·11	0 ·35	—	—	—	9 ·08	88 ·06	2 ·86	Right jugular into heart	124	Very steady good respiration.
XXXV*a*	—	—	—	78 ·22	47 ·27	8 ·06	0 ·31	—	—	—	9 ·00	87 ·7	3 ·30			
XXXVI	3 ·3		21 ·5	80 ·15	71 ·55	8 ·02	0 ·58	94 ·5	17 ·54	45 ·7	34 ·86	60 ·12	5 ·03	Inferior vena cava	130	Respiration, 40 per min. Some mucus in trachea, gurgling breathing, slow and shallow.

The first nine experiments were made some time ago, without the precautions we have insisted upon as being necessary in the use of the blood pump, and the nitrogen values are above the true value. The last two experiments given in Table III were carried out with all precautions, the pump washed out with oxygen, and rendered vacuous with liquid air, and *it will be seen that the nitrogen values are* 1·07 *and* 0·96 *per cent. of blood respectively;* figures exactly the same as those which we found for the nitrogen-content of cat's blood.*

The oxygen-content of venous blood is seen to vary from 2·63 c.c. to 10·8 c.c. per 100 c.c. of blood.

In Table IV we give the values of venous blood of cats breathing oxygen. The oxygen figures vary from 3·5 c.c. to 13·49 c.c. per 100 c.c. of blood, and are of much the same order as during the respiration of air.

Conclusions.

The experiments detailed in this paper would appear to justify the following conclusions:—

1. The inhalation of oxygen does not materially augment the quantity of this gas in the blood.

2. The inhalation of oxygen apparently does not alter the average carbon dioxide content of the blood.

We are carrying out further experiments on the inhalation of oxygen, with a view to testing the validity of the above conclusions.

We take this opportunity of thanking the Committee of the Government Grant of the Royal Society for assistance in partly defraying the expenses connected with this work.

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* 'Journ. Physiol.,' 1910, vol. 41, p. 61.

The Physiological Effects of Low Atmospheric Pressures, as Observed on Pike's Peak, Colorado. (Preliminary Communication.)

By C. Gordon Douglas, B.M., Fellow of St. John's College, Oxford; J. S. Haldane, M.D., L.L.D., F.R.S., Fellow of New College, Oxford; Yandell Henderson, Ph.D., Professor of Physiology, Yale University Medical School; and Edward C. Schneider, Ph.D., Professor of Biology, Colorado College.

(Received January 11,—Read January 18, 1912.)

The following is a short preliminary account of a series of observations made in the summer of 1911 on the summit of Pike's Peak, Colorado.

Pike's Peak is 14,109 feet above sea-level, the barometric pressure on the summit being about 18 inches (457 mm.). There is an excellent stone house close to the summit, in which we were accommodated during our stay of five weeks. The main object of the expedition was to discover to what extent, and by what means, adaptation takes place to low barometric pressure and consequent deficiency in the partial pressure of oxygen in the air.

Our chief conclusions are as follows:—

(1) After two or three days on the summit of Pike's Peak very distinct signs of acclimatisation began to appear.

(2) Before acclimatisation occurred blueness of the lips and face, nausea, intestinal disturbance, headache, fainting in some persons, and periodic breathing were observed, besides great hyperpnœa on exertion or holding the breath for a few seconds.

(3) All these symptoms are referable, directly or indirectly, to want of oxygen, produced by the diminished partial pressure of oxygen in the air. We did not observe, either in ourselves or in the large number of persons who ascended the Peak, any symptoms (apart from the effects of the bright light) not referable to the same cause.

(4) After acclimatisation had occurred these symptoms disappeared, with the exception that hyperpnœa on exertion or on holding the breath for a few seconds was still much greater than usual. Periodic breathing was still observed occasionally, and blueness of the lips and face was present after continuous and fairly powerful exertion, such as walking up hill.

(5) The respiratory exchange during rest remained about normal in the one subject on whom exact experiments were made, and the respiratory exchange during work did not appear to be markedly increased.

(6) After acclimatisation the alveolar carbon dioxide pressure was diminished from about 40 mm. to about 27 mm. during rest or moderate exertion, which corresponded to an increase of about 50 per cent. in the ventilation of the lung alveoli. During severe exertion the alveolar carbon dioxide pressure was about half what it normally is during similar exertion, which corresponded to an increase of about 100 per cent. in the hyperpnœa ; and owing to a temporary alteration in the respiratory quotient the breathing was still further increased.

(7) The change in the level of alveolar carbon dioxide pressure occurred gradually after going up, and disappeared gradually on coming down, the change taking a number of days to reach completion.

(8) The percentage of hæmoglobin in the blood increased for several weeks on the summit of Pike's Peak, and varied in different acclimatised persons from 115 to 154 per cent. on the scale of the Gowers-Haldane hæmoglobinometer, corresponding to an oxygen capacity of from 21 to 28·5 c.c. of oxygen per 100 c.c. of blood. The number of red corpuscles per cubic millimetre of blood increased parallel with the hæmoglobin, and the percentage volume of red corpuscles, as determined by the hæmatocrit, also increased in proportion to the percentage of hæmoglobin.

(9) A large increase in the total amount of hæmoglobin (determined by the carbon monoxide method) in the body occurred during the first three weeks, and along with this increase there was found, except in the first week, a slight increase in blood volume, as well as the increase, already referred to, in the percentage of hæmoglobin.

(10) On coming down from Pike's Peak the hæmoglobin percentage diminished much more rapidly than the total hæmoglobin, so that the blood-volume was still further increased at first. It required about four weeks for the excess of hæmoglobin and blood-volume to disappear, though the hæmoglobin percentage fell to normal much earlier.

(11) So far as we could ascertain, there was very little change in the rate of circulation on Pike's Peak after acclimatisation. Pulse and blood-pressure were but little affected. In most cases, however, there was a slight increase in the pulse rate.

(12) After acclimatisation the oxygen pressure in the arterial blood (measured by the carbon monoxide method) rose during rest to about 35 mm. of mercury above the alveolar oxygen pressure (66 per cent. higher), and remained at a level of only about 12 mm. below the normal oxygen pressure at sea-level. Immediately after ascending the Peak and before acclimatisation had occurred, the arterial oxygen pressure was found to be about 45 mm. below normal, and only slightly above the alveolar oxygen pressure. This change

appears to be due to a progressive increase in the activity of the alveolar epithelium in secreting oxygen inwards. On raising the alveolar oxygen pressure to normal, the difference between alveolar and arterial oxygen pressure diminished rapidly.

(13) Acclimatisation to high altitudes is due mainly to the increased secretory activity of the alveolar epithelium, but partly also to the increased lung ventilation, and to a lesser extent to the increased hæmoglobin percentage in the blood. The acclimatisation takes some days to develop. During rapid ascents in balloons or aeroplanes it would not have time to develop, and this explains the contrast between the experience of balloonists, etc., and that of mountaineers who ascend gradually.

The Development of a Leucocytozoon of Guinea-Pigs.

By Edward Halford Ross, M.R.C.S. England, L.R.C.P. London.

(Communicated by Sir Ronald Ross, K.C.B., F.R.S. Received January 23,—Read February 29, 1912.)

(From the Laboratories of the McFadden Research Fund, Lister Institute.)

[Plate 1.]

The presence of "bodies" within the large mononuclear leucocytes of guinea-pigs was first noticed by Kurloff (1898). He described them as inclusions; for in a drop of guinea-pig's blood he noted that many of the large lymphocytes contained, within their cytoplasm, clear, spherical vacuoles which were distinct from the nucleus, and which had not been described before; and he suggested the possibility of these bodies being accessory nuclei. Since their discovery by Kurloff they have been subjected to much research; and papers describing various observations concerning them have been published by Burnett (1904), Staubli (1905), Goldhorn (1905), Ledingham (1906), Howard (1907), Pappenheim (1908), Patella (1908), Hunter (1909), and Schilling (1911).

Kurloff noticed that when the blood containing these bodies was fixed and stained, they contained a nucleus-like structure staining with nuclear dyes, but he believed them to be vacuoles formed by a secretion product of the cells which held them. Ehrlich (1906) also thought that Kurloff's bodies represented some "Secretstoff." Dr. Ledingham, to whom I am indebted for much information, seems to have been the first to suggest the possibility of their parasitic nature, and he mooted an analogy to the *Cytorryctes variolæ* or *vaccinæ*. Goldhorn (1905) boldly called them leucocytozoa. The most recent work published on the subject is that of Schilling (1911). He has examined these bodies by "vital" staining with Azur, and he has described some of the earlier stages of their development while in the mononuclear leucocytes (lymphocytes). He believes that the rod stage precedes the granule stages, and this has caused him to adhere to the opinion that Kurloff's bodies must be classed with the Chlamydozoa, symbiotic structures, or vaccine inclusions.

Early in 1911, while examining a guinea-pig's blood by a new jelly method of examination of blood cells, H. C. Ross saw Kurloff's bodies, and pointed out to me that the method demonstrated the probability of their parasitic nature. The new method, which was devised partly at the suggestion of Sir Ronald Ross, K.C.B., has already been fully described (H. C. Ross, 1909); the bodies then seen were in the earlier stages of their development. But the inclusions stood out so clearly by this method that I determined to continue the observations, for this technique seemed to show details of structure which had not been described before; and since by the new process the bodies can be subjected to the action of various stains and chemical agents there was a possibility of the phases of their development being observed. I may state that I have now been able to convince myself that these bodies are living parasites of the mononuclear white corpuscles (lymphocytes), and henceforth in this paper I propose to call them such.

I use a modification of the original jelly method—it is as follows:—A 2-per-cent. solution of agar in water is boiled, sterilised and filtered. To 5 c.c. of the filtrate is added 0·5 c.c. of a 10-per-cent. solution of sodium chloride in water, and 0·5 c.c. of a 1-per-cent. solution of Azur II in water. The total bulk of the mixture is made up to 10 c.c. in a test-tube. When molten, a small quantity of the jelly is allowed to spread itself in a thin film on a microscope slide and to cool and set. Then a drop of guinea-pig's blood (or citrated blood) containing Kurloff's bodies (about 90 per cent. of the guinea-pigs examined by me, and which were obtained from dealers in England, are infected) is placed upon a cover-glass, and this is inverted on to the set jelly. The blood spreads out between the cover-glass and the surface of the jelly, and, after an interval of five minutes, during which the blood corpuscles come to rest, the specimen may be examined under the higher powers of the microscope. After a further interval of a few minutes—the exact period varying slightly with the temperature of the room—the granules of the leucocytes begin to stain, after which their nuclei gradually stain a deep blue; the contours of the erythrocytes, as well as those of the leucocytes, show up clearly, and the method is a pretty example of *in vitro* staining. In some of the larger mononuclear cells the colourless parasites will be noticed at one side of the protoplasm. These parasites are inside the cell, because the shape of the nucleus of the lymphocytes is moulded according to the size of the parasite, which grows larger as it develops—in its youngest stages it is small, while in its last intracellular stages it bulges the lymphocyte cell wall and squeezes the nucleus into a small space; this point is of interest because, as Hunter has shown, Patella claimed that Kurloff's bodies lie upon and not in the lymphocytes. In cells containing the larger parasites smaller vacuoles can also be seen; these latter always remain clear and transparent even when examined on stain-containing jellies, and they vary in numbers, and slightly in size, in different examples. It has been suggested that these smaller, subsidiary vacuoles are polar bodies, but more probably they contain excretory products of the lymphocytozoa into the cytoplasm of their hosts, for they become larger and more numerous as the parasite grows.

When examined on the jelly, and immediately before the staining of the nucleus of the leucocytes, the contents of the parasites begin to stain*—the internal chromatin structure of the spherical sac embedded in the lymphocytes' cytoplasm becomes purple and remains stained for several hours, so that its examination is readily made. If the bloods of a number of infected guinea-pigs are watched in this manner from day to day what appear to be the successive stages of the growth of the parasite in the lymphocyte can be seen and drawn; but the leucocytes of a single animal at any particular moment contain, usually but not always, parasites in the same stage of development. The cycle, however, can be followed by observing the blood of one guinea-pig hourly.

The interpretation which I place upon the appearances I have seen are as follows:—The parasite presents itself, in the smallest phase of its intracorpuscular cycle, as a tiny translucent body embedded within the cytoplasm of the larger mononuclear blood corpuscles and near the periphery of those cells. Usually one of such bodies is present in any one cell, but occasionally two or even three parasites may occur in the same cell. The parasite, in this early stage, contains a double purple dot (figs. 1, 2); in this phase it resembles the Leishman-Donovan bodies found in human leucocytes in cases of Kala Azar. When first seen the dot is motionless, but after a time on the jelly, as the lymphocyte host becomes disorganised, it may show some Brownian movement. In the next stage the parasite is larger, and the chromatin dot has divided into two or more dots until the sphere-like sac may be packed with them (fig. 3). Then each dot becomes dumb-bell shaped (fig. 4), and again, by a simple process of elongation, rod shaped (figs. 5, 6, 7). The parasite may contain one of these rods (fig. 9), or it may be full of them—the actual numbers varying in different examples. Sometimes a parasite may contain one or more rods, some dumb-bells, and some dots. But the size of the parasites increases steadily with these successive stages of the development of their contained chromatin (compare figs. 1 and 15). During the rod formation, the smaller subsidiary vacuoles already mentioned appear in the cytoplasm of the host cell (figs. 3, 5, 12); they never contain any chromatin and remain unstained. With its growth the parasite begins to compress the nucleus of the lymphocyte (figs. 13, 14), and the wall of the latter can be seen as a shell enclosing the parasite (figs. 14, 15, 16). The rods grow longer and thicker (figs. 8, 9, 10) until they stretch across the parasite, and their ends may be doubled against its wall, and they may then present in optical section an erroneous impression of flattening or a terminal bulging (figs. 8, 13, 14). In the next stage a stout flagellum grows out from both ends of the rod (figs. 8, 11, 12, 13), which becomes rolled up in a coil within the sphere (figs. 13, 14, 15). The rod with its two flagella splits longitudinally in its whole length (figs. 8, 12), and this process of splitting takes place again and again. The fission throughout is always lengthwise, never transverse. A specimen in this stage will show the parasite, now equal in size to the original dimensions of its host-cell, bulging the wall of the latter, compressing the nucleus into a small space, and containing within its interior a mass of woven, twisted, and intertwined purple threads, a conglomerate maze of worm-like spirilla stained red by the Azur dye (figs. 15, 16).

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* It must be emphasised that if the jelly contains excess of salts or impure stains, the wall of the parasite will stain in an irregular manner, and then patches of stain will hide its contents. Furthermore, if the blood on the jelly dries, or if the blood is fixed in any way, the same thing occurs. Similarly, patchy staining is obtained by the various fixed film methods in vogue, as, for example, Romanowsky's or Jenner's stains. Even Azur stain, when applied to the dried or fixed films of blood, will not demonstrate the details of the development of the parasite. No alkali should be added to the jelly.

Arrived at its maturity, the parasite breaks away from the shell of its host-cell and then bursts, setting free the threads into the plasma (fig. 17). But the flagellate forms, owing to the fact that they are stained, are dead and motionless, and they may remain attached to the shrunken sphere sac, their ends waving in the currents set up.

It was found very difficult to demonstrate the motile, flagellate forms of the parasite when free in the blood. They cannot be seen then by the jelly method, because, probably, they stain momentarily as the trypanosomes do, and immediately die and become achromatic, and unless stained they are not visible. By the examination of ordinary wet films of the blood I was unable to demonstrate the presence of these free flagella, although a disturbance of the corpuscles was frequently seen. But the blood of some infected guinea-pigs, drawn under all aseptic precautions and examined by the dark ground illumination, showed free-swimming spirochæte-like bodies. It was not until the blood of highly infected guinea-pigs containing full matured lymphocytozoa was treated with an equal part of a 1-per-cent. solution of "globin"* and incubated at 37° C. for eight hours that the free flagellate forms in the blood plasma could be fixed and stained by ordinary methods (fig. 18). Even by this process it is not always possible to demonstrate them, and the maceration involved gives them the appearance of spirilla with blunt ends. However, some of the spirilla obtained after the treatment with the "globin" show the wavy outline of spirochætes. Sir Ronald Ross was the first to suggest that these flagellate forms constitute the gametes of the parasite; this seems quite probable, though no separate female form has yet been noticed. It will be remembered that Lewis suggested that trypanosomes are sperms, and, perhaps, these spirochæte-like bodies are similar stages of a larger parasite.

What may possibly be the last phase of this parasite has occasionally been seen in preparations which had been submitted to the action of "globin" for a further period of four hours. It is an object which resembles somewhat the trypanosome "latent bodies" described by Moore and Breinl (fig. 19). Hunter has also mentioned the presence of amœboid forms of this parasite being free in the plasma, but he does not picture them. These may be the form now drawn (fig. 19).

Dr. J. W. Cropper and I have repeated and can confirm the experiments of Ledingham (1906) and Hunter (1909), namely, that newly-born guinea-pigs do not show these lymphocytozoa in their blood. Although a pregnant animal may be markedly infected, the young, when born, possess no parasites. As has been already observed by these writers and by Schilling (1911), the number of parasites found in both the peripheral blood and in that of the internal organs of any one infected guinea-pig varies greatly from day to day. The parasites seem to appear in large numbers, to diminish, to disappear, and then, after a varying period of time, to reappear. Except for a slight anæmia, shown by the presence of an increased number of erythroblasts in the peripheral blood, the guinea-pigs do not suffer apparently. The livers of many of these infected animals show, however, single or multiple white patches of necrosis varying in size between that of a pin's head to that of a large pea, and extending into the substance of the organ. But we have no proof, as yet, of their direct relation to the parasite.

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* The filtrate of a solution of hæmoglobin which has been precipitated by heat H. C. Ross claims that this substance induces the division of certain cells.

Fixed specimens of the various stages of the development of this parasite may be made by substituting an equal amount of a 1-per-cent. solution of caustic soda in the jelly for the sodium chloride solution. By this means the red blood corpuscles are laked, but the nuclei of the leucocytes and the chromatin of the lymphocytozoa stain well. The cover-glass can then be lifted from the jelly and mounted while still wet in Canada balsam. Many of the leucocytes with the contained parasites will adhere to the cover-glass and will retain their stain.

Since writing this paper, Hindle has published a preliminary note (Hindle, 1911), "On the Life-cycle of *Spirochæta gallinarum*." He asserts that these spirochætes possess an intracellular stage within the cells of the Malpighian tubes of the tick, *Argas persicus*. In view of the life-history of this lymphocytozoon of guinea-pigs his work is of great interest.

I have to express my indebtedness to Dr. J. W. Cropper and to Dr. H. Bayon for their help in these researches; the latter was the first to recognise the free swimming, spirochæte-like bodies. I also wish to thank Prof. Minchin and Dr. Martin for much help and advice and the interest they have taken in this work.

Summary.

Kurloff's bodies are parasites, lymphocytozoa inhabiting only the mononuclear cells of the guinea-pig's blood.

These lymphocytozoa have an intracorpuscular stage, and ultimately give rise to free swimming, spirochæte-like bodies, which may be gametes.

The development of the spirochæte-like body is demonstrated.

The name *Lymphocytozoon cobayæ* is suggested for this parasite.

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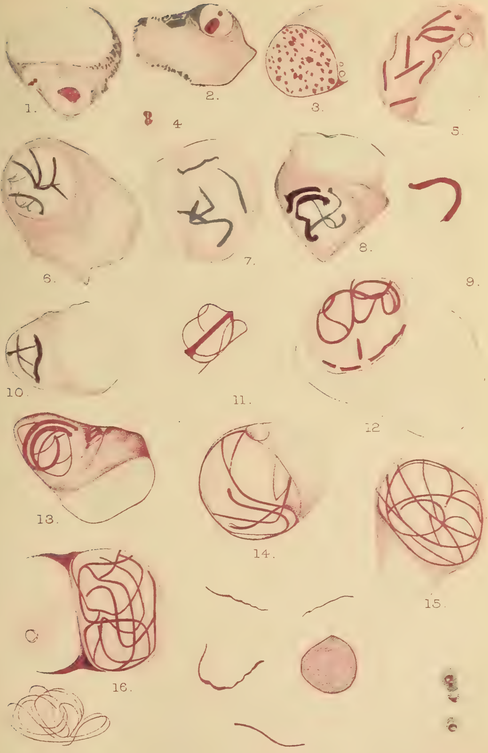
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DEVELOPMENT OF A LEUCOCYTOZOON OF GUINEA-PIGS.

The Bacterial Production of Acetylmethylcarbinol and 2.3-Butylene Glycol from Various Substances.—II.[*]

By Arthur Harden, F.R.S., and Dorothy Norris, Biochemical Department, Lister Institute.

(Received January 25,—Read February 29, 1912.)

The action of *B. subtilis* (Cohn), *B. mesentericus vulgatus* (Flügge) (*B. vulgatus* (Flügge) Migula), and *Tyrothrix tenuis* (Duclaux) (*B. tenuis* (Duclaux) L. and N.) on various substances has been investigated by Péré (1). This observer, on distilling his various culture media, obtained lævo-rotatory distillates strongly reducing to Fehling's solution. In all cases he concluded that the volatile substance present was glyceraldehyde, and upon his results based a theory that sugars undergoing bacterial fermentation break down primarily to a triose, that is to say, glycerose.

He was, however, unable to characterise his compound satisfactorily; for example, it did not give Schiff's reaction, no osazone was obtainable, and although in some cases he obtained small quantities of lead, calcium, and barium salts of an acid formed by the oxidation of his volatile substance with nitric acid, which he took for salts of glyceric acid, the quantities analysed were so small that no reliance can be placed upon the results. Moreover, none of his salts was obtained in crystalline form.

Soon after the appearance of Péré's work Wohl (2) succeeded in preparing glyceraldehyde in a pure state, and found that it was non-volatile in steam, that it gave Schiff's reaction, and also formed a highly characteristic osazone, M.P. 131° C. It seemed therefore impossible that the volatile substance obtained by Péré could have been glyceraldehyde, and a further investigation of the subject has therefore been made.

In a previous communication (3) the action of *B. lactis aërogenes* and *B. cloacæ* on many carbohydrates, alcohols, etc., has been described. With glycerol itself the liquid obtained on distilling the culture medium was absolutely without reducing power. In the majority of cases, however, the distillate possessed reducing power which was shown to be due not to the presence of glyceraldehyde, but to that of acetylmethylcarbinol, $CH_3{\cdot}CH(OH){\cdot}CO{\cdot}CH_3$, the substance responsible for the Voges and Proskauer reaction (4), which had previously been observed as a product of the bacterial fermentation of glucose by Grimbert (5). The above experiments were carried out under anaërobic conditions, but Walpole (6) has shown that the yield of carbinol may be increased by aërobic culture. The whole of Péré's experiments were carried out under aërobic conditions, and although he employed different organisms from those given above, they have all been shown by Desmots (7) to be capable of producing acetylmethylcarbinol under suitable circumstances. Desmots, in fact, describes experiments very similar to those carried out by Péré, but makes no mention of the possible formation of glyceraldehyde. It seems therefore not at all unlikely that Péré's volatile reducing substance was acetylmethylcarbinol and not glyceraldehyde. His experiments have accordingly been repeated, using identical culture media and conditions of growth, and the results of these investigations form the subject of the present communication. In addition to repeating Péré's experiments a quantitative estimation of the action of *B. lactis aërogenes* on glycerol has been made. In this case neither acetylmethylcarbinol nor glyceraldehyde is obtained.

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[*] For first part see paper read February 1, B, vol. 84. p. 492.

Action of B. subtilis *and* B. mesentericus vulgatus *on Mannitol and* Tyrothrix tenuis *on Glucose.*

The culture media were made up exactly in the same way as those used by Péré, and in the case of the first two organisms named consisted of 20 grm. of mannitol in 200 c.c. water containing 2 grm. ammonium phosphate, 1 grm. ammonium sulphate, and 0·4 grm. potassium phosphate. For the experiment with *Tyrothrix tenuis* 5 grm. of glucose were made up to 100 c.c. with broth. After sterilisation and inoculation with the organism in question, the various culture media were incubated at 37° C. In every case growth was continued for 30 days, after which time the cultures were worked up according to Péré's directions. To take one example—the action of *B. subtilis* on mannitol—after the 30 days' incubation the culture medium was made acid with citric acid and distilled, the distillate was found to be strongly reducing and lævo-rotatory, and also gave a very strong Voges and Proskauer reaction, which is characteristic of acetylmethylcarbinol, but is not given by glyceraldehyde. The remaining distillate was then made alkaline and again distilled, yielding a second time a reducing lævo-rotatory body giving the Voges and Proskauer reaction. This second distillate was then steam-distilled for three hours, and by the end of that time the whole of the reducing body had passed over with the steam, the residue being non-reducing and optically inactive. The steam distillate, on the other hand, was still lævo-rotatory, reducing, and gave the Voges and Proskauer reaction. An osazone was prepared from this distillate and gave a definite melting point of 243° C., corresponding to the phenylosazone of diacetyl, which is always obtained from acetylmethylcarbinol in this manner. In a similar way the action of

B. mesentericus vulgatus and *Tyrothrix tenuis* on mannitol and glucose respectively was also examined and similar results obtained.

The Action of Tyrothrix tenuis *on Glycerol.*

This offered a case of much greater interest, as the formation of acetylmethylcarbinol would involve a carbon synthesis, and does not take place from this substance when *B. lactis aërogenes* is used under anaërobic conditions.

Péré's directions were again carefully followed, 5 grm. of glycerol were made up to 100° C. with broth, and, after sterilisation and subsequent inoculation, were incubated at 37° C. for 30 days. An investigation of the culture medium, as described above, again showed the presence of acetylmethylcarbinol, which passed over into the distillate, and was characterised by the preparation and analysis of the osazone, as well as by the Voges and Proskauer reaction. The lead, calcium, and barium salts of the oxidation products analysed by Péré, and believed by him to be the salts of glyceric acid, were in all probability the salts of lactic acid. As he was only able to analyse extremely small quantities (0·032 grm. of a lead salt containing 50 per cent. Pb) of non-crystalline substances, the experimental error was probably too great for him to distinguish between these two acids. There is therefore no evidence to show that glyceraldehyde is produced in the above fermentations, and the theory that sugars undergoing bacterial fermentation are first broken down to trioses derives no support from this investigation.

The Action of B. lactis aërogenes (*Escherich*) *on Glycerol under Anaërobic Conditions.*

The experiments previously described were all carried out under aërobic conditions, and, as an example of anaërobic decomposition, the action of *B. lactis aërogenes* on glycerol has been studied. This organism under these conditions forms acetylmethylcarbinol from all the hexoses, but produces none from glycerol, no reducing substance at all being found among the products of the reaction.

The method of investigation was substantially that used by Harden (8), the gases, however, being collected and measured in the apparatus devised by Harden, Thompson, and Young (9). The organism was grown in an atmosphere of nitrogen, the medium consisting of 1 per cent. Witte's peptone solution containing 10 per cent. of glycerol, sufficient chalk being added to neutralise the acids formed during fermentation. In the glycerol experiments quoted, the times of growth were respectively five weeks, two weeks, and four weeks. A different strain of the organism was used in Experiment 1 from that used in Experiments 2 and 3.

The glycerol added, and the amount unfermented, were estimated by means of the method of Zeisel and Fanto (10), as described below.

Estimation of the Residual Glycerol.—100 c.c. of the liquid in which the organism had grown were evaporated at a low temperature under reduced pressure, in order to ensure the removal of all alcohol. The residue was then taken up in a small quantity of water, the volume made up to 100 c.c. with water, and 5 c.c. of this solution taken for the estimation.

Search for Acetylmethylcarbinol.—A portion of the culture medium was distilled and the distillate tested for reducing power with Fehling's solution. In every case the distillate was found to be non-reducing and the absence of acetylmethylcarbinol was further confirmed by trying the Voges and Proskauer reaction, which was invariably negative. It was also found impossible to prepare any osazone.

Detection of 2.3-Butylene Glycol.—This substance was detected and estimated as previously described, the estimation being made on 300 c.c. of the culture medium in which the organism had been grown. In every experiment this glycol was found to be present, the other products being ethyl alcohol, formic, lactic, and succinic acids, carbon dioxide, and hydrogen.

The diacetylphenylosazone prepared from the oxidation product of the butylene glycol was analysed and gave the following results:—

0·1372 grm. substance gave 0·3656 grm. CO_2 and 0·0876 grm. H_2O. $C = 72·6$ per cent.; $H = 7$ per cent.

0·0950 grm. substance gave 17·5 c.c. N at 21° C and 765·5 mm. $N = 21·12$ per cent.

$C_{16}H_{18}N_4$ requires $C = 72·2$ per cent., $H = 6·8$ per cent., $N = 21·05$ per cent.

The lactic acid produced was also characterised by the preparation and analysis of the zinc salt and by Fletcher and Hopkins' reaction (11).

0·1500 grm. of the zinc salt dried at 105° C. gave 0·0499 grm. ZnO. $ZnO = 33·28$ per cent.

$(C_3H_5O_3)_2Zn$ requires $ZnO = 33·4$ per cent.

The analysis of the calcium salt prepared from the succinic acid formed gave the following results:—

0·0550 grm. substance gave 0·0202 grm. CaO. $CaO = 36·7$ per cent.

$(C_4H_4O_4)Ca$ requires $CaO = 35·9$ per cent. The pure acid was also isolated in this case and gave M.P. 183–4° C.

The percentage of these substances on the weight of glycerol fermented is shown in the following table, Columns 1, 2, and 3. Columns 4 and 5 show for comparative purposes the result of the action of *B. lactis aërogenes* on glucose and mannitol respectively.

Table I.

	Glycerol.			Glucose.	Mannitol.
	1.	2.	3.	4.	5.
Alcohol	35·2	37·7	37·3	17·1	32·5
Acetic acid..............	6·1	5·0	7·3	5·1	2·5
Lactic acid..............	13·0	12·7	11·13	5·5	8·6
Succinic acid	4·05	1·6	4·03	2·4	3·2
2.3-Butylene glycol ...	9·9	Not estimated		(27·2)	(12·0)
Formic acid	6·38	4·9	7·5	1·0	1·5
Carbon dioxide	22·4	28·37	31·8	38·0	35·5
CO_2, c.c. per grm.......	110·6	144·0	160·7	198·3	180·3
H_2, c.c. per grm.	79·8	139·0	156·7	82·4	138·3
Ratio H_2/CO_2...........	0·72	0·97	0·97	0·42	0·77

The figures in brackets are estimated from other experiments.

Table II shows the number of carbon atoms per molecule of glycerol decomposed, represented by each product.

Table II.

	Glycerol.			Glucose.	Mannitol.
	1.	2.	3.	4.	5.
Alcohol	1·42	1·43	1·49	1·34	2·57
Acetic acid..............	0·18	0·15	0·22	0·31	0·15
Lactic acid..............	0·40	0·39	0·34	0·33	0·52
Succinic acid	0·12	0·05	0·12	0·15	0·20
2.3-Butylene glycol ...	0·39	Not estimated		(2·17)	(0·97)
Formic acid and CO_2	0·58	0·69	0·82	1·64	1·53
Total	3·09	2·71	2·99	5·94	5·94
Hydrogen, atoms per molecule	0·65	1·13	1·28	1·33	2·26

Columns 4 and 5 are again comparative ones of *B. lactis aërogenes* on glucose and mannitol.

It is interesting to find that alcohol accounts for 35·2 per cent. of the glycerol used, as against 17·1 per cent. in the case of glucose. Harden suggested (8) that the source of the alcohol might be the presence in the molecule undergoing decomposition of the terminal group $CH_2(OH)\cdot CH(OH)$–. This was confirmed in the case of glucose and mannitol, this latter substance yielding twice the amount of alcohol produced under similar conditions from glucose. It would be interesting to find whether the same relationship holds in the case of glycerol and glyceraldehyde. Formic acid (or its decomposition products, CO_2 and H_2) and alcohol, which might be formed according to the equation

$$C_3H_8O_3 = C_2H_6O + CH_2O_2,$$

make up 64—77 per cent. of the glycerol fermented.

Summary.

1. The volatile reducing substance obtained by Péré in the aërobic fermentation of mannitol by *B. subtilis* and *B. mesentericus vulgatus*, and of glucose and glycerol by *Tyrothrix tenuis*, is acetylmethylcarbinol, which is readily volatile in steam, gives the Voges and Proskauer reaction, and forms the phenylosazone of diacetyl.

2. The action of *B. lactis aërogenes* on glycerol, under anaërobic conditions, does not give rise to any reducing substance.

The products of this decomposition have been quantitatively estimated and are as follows:—Ethyl alcohol, formic, acetic, lactic and succinic acids, carbon dioxide, hydrogen and 2.3-butylene glycol.

[*Note added February* 29, 1912.—Since writing the foregoing paper our attention has been called to a paper by Fernbach* in which he shows that *T. tenuis* acts both on glucose and glycerol with the production of non-volatile dihydroxyacetone. Volatile reducing substances were also formed which he regards as a mixture of methylglyoxal and formaldehyde. Since neither of these substances is optically active they cannot be identical with the lævo-rotatory substance obtained by Péré and ourselves, so that Fernbach's observations in no way disprove our conclusion that the optically active, volatile substance produced is acetylmethylcarbinol.]

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(10) Zeisel and Fanto, 'Z. anal. Chem.,' 1903, vol. 42, p. 551.
(11) Fletcher and Hopkins, 'Journ. Phys.,' 1907, vol. 35, p. 247.

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* 'Compt. Rend.,' 1910, vol. 151, p. 1004.

On the Distribution of the Nerves of the Dental Pulp.

By J. Howard Mummery.

(Communicated by Prof. J. Symington, F.R.S. Received November 6, 1911,—
Read February 1, 1912.)

(Abstract.)

The mode of innervation of the dentine of the human tooth has long been a matter of controversy; while clinical evidence is strongly in favour of a nerve supply to this tissue, the difficulties met with in tracing nerve fibres in such a difficult substance to examine, as the dentine, has been a very considerable hindrance to the investigation. It has been very difficult to account for the passage of such very acute sensation from the periphery of the dentine in the absence of nerve fibres in that situation, and I have long felt, with others, that sensation in the tooth would be found to be conducted by nerve fibres, as in other tissues of the body. As long ago as 1891 I made preparations which appeared to show that nerve fibres from the pulp entered the dentine, but, by the iron and tannin impregnation process I then employed, could not satisfactorily demonstrate it.

During the last year I have, I think, with several methods of preparation, been successful in making it fully evident that the dentine is richly supplied with nerves from the pulp, which do not terminate, as has been hitherto generally supposed, at the inner margin of the dentine, but enter the tubules of that tissue and traverse them to their peripheral terminations at the enamel and cementum margins.

The bundles of medullated fibres which enter the tooth at the apical foramen traverse the pulp in more or less parallel lines, running in most cases in company with the blood-vessels. They send off numerous side branches, which at the periphery of the pulp lose their medullary sheath, the axis cylinders spreading out into a mass of neurofibrils which enter into a more or less dense plexus beneath the odontoblast layer of cells. From this plexus, known as the plexus of Raschkow, fine neurofibrils pass between and around the odontoblasts, enclosing them in a fine meshwork, and enter into a narrow plexus at the inner margin of the dentine. This has usually been described as the mode of termination of the nerve fibres of the pulp, but fibres can be seen arising from this plexus, which might be better termed the "marginal plexus," and passing into the dentinal tubes.

These neurofibrils pass into the dentine in great abundance and seem to be equally distributed in the coronal portion and considerably below the neck of

the tooth, but become more and more scattered as they approach the apex of the root. This is especially well shown by Ramón y Cajal's silver nitrate process. The most successful preparations have been those prepared with silver nitrate and with gold chloride, but the erratic manner in which these substances select the tissues is well known, and it is only here and there among some hundreds of sections that a thoroughly successful impregnation is found.

In this investigation I have made use of fresh calcified teeth ground on a lathe, of teeth decalcified with nitric acid, and with formic acid, and of calcified teeth ground on a stone after impregnation with balsam by the Weil process. This latter method appears to me superior to all others, although a very tedious and troublesome one to carry out successfully; good preparations fully repay the trouble; there appears to be no shrinking of the cells, such as occurs in specimens decalcified with acids, and the matrix of the dentine is not stained. The staining of the matrix in decalcified specimens greatly interferes with the clear observation of the contents of the tubules, the minute longitudinal striation seen in the matrix, especially in silver preparations, giving rise to very deceptive appearances. In a well impregnated balsam preparation the contents of the tubules are stained with the silver, the surrounding matrix remaining quite clear and unstained.

I have also procured some very successful slides by staining a small piece of a calcified tooth in bulk with a nuclear stain, passing it through the nitrate of silver process, and then decalcifying with formic acid.

In the substance of the dentine in well impregnated preparations fine beaded fibres can be traced in the tubules, and in the majority of cases there appear to be two fibres in each tubule which can be traced in many preparations to the inner margins of the enamel and cementum.

A Confusion-Test for Colour-Blindness.

By George J. Burch, M.A., D.Sc. Oxon, F.R.S.

(Received December 22, 1911,—Read March 28, 1912.)

The work described in this paper was done in conjunction with Lieut.-Col. W. R. L. Scott, late of the 62nd Foot, who was very nearly, but not quite, red-blind. He could not under ordinary circumstances distinguish the red coat of a soldier from the black coat of the civilian, though on closer inspection it seemed "not so good a black." His colour-sensations are given in my paper on "Artificial Temporary Colour-Blindness."*

He continued to take an active interest in the subject, and gave me a good deal of help from time to time in testing the colour-sensations of other people, in which he was very successful, his own colour-blindness enabling him to win their confidence. It had been intended that his name should appear as joint-author of this paper, but he died on April 9, 1911, aged 78.

In the summer of 1903 we arranged to collaborate in painting a series of test-cards, accurately adjusted to his colour-sensations, oil-colours being selected, as easier of manipulation than water-colours, and less likely to fade.

I. For the first, I painted the half of an Academy board, 9 inches by 7, with two coats of vermilion, using very little medium and painting as solidly as possible. When this was dry, several different kinds of green were mixed, spread on strips of wood, and held near the red background, the most satisfactory being selected by Lieut.-Col. Scott, who indicated which of the colours on the palette might improve it. This I tried until he expressed himself completely satisfied with the match. My function was to make the colours as unlike as possible to normal eyes, and his to make them match perfectly to the red-blind. The other half of the board was then painted with the mixture, which was about the colour of a year-old ivy leaf, and it was allowed a fortnight in which to dry and also to see if any change of colour occurred on drying.

I then painted, in his absence, on the green part of the card the words DON'T GO, the letters N'T being in vermilion and the rest in blue. The blue he could see immediately—in fact there seems to have been to him as much difference between green and blue as there is to us between red and green. But he read the inscription as DO GO, being absolutely unable to distinguish the letters in vermilion. After a while, by holding the panel sideways, so as to get the light on the brush-marks, he managed to decipher them, but he could see no difference in colour. In order to prevent the brush-marks from being seen, I devised the plan which constitutes the novelty of this test.

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* 'Phil. Trans.,' B, vol. 191, p. 29.

The card lies at the bottom of a box like that of an old-fashioned stereoscope, but larger. In front of the card, at a distance of 6 or 8 inches from it, I fix a sheet of perforated zinc, and the observer looks through a short tube in the top of the box, containing a convex lens of about 8 diopters, focussed on the zinc. The card is so far beyond the zinc that it is necessarily out of focus, even for those possessing full power of accommodation, all that can be seen through the holes being merely the colours, which form a mosaic of coloured dots, not unlike those of Prof. Stilling's test, from which, in fact, I got the idea.

But the method has this advantage over Stilling's cards—given the colours, any design can be painted in a few minutes. It is astonishing how soon the exact description of a test-card becomes known if many people from the same neighbourhood are tested; hence the importance, even in the absence of any conscious attempt to deceive the examiner, of being able to vary the lettering.

A year later, Lieut.-Col. Scott said that the colours were no longer a match, and he could even read the red letters through the perforated zinc. On close inspection there appeared to be a sort of whitish bloom on the surface of the vermilion, but so very slight that few people would have noticed it. He found that fresh vermilion still matched the green, as did the old after the application of oil. From this it would appear that the green was unchanged. The whitish bloom would represent to him an addition of blue, to which he was exceedingly sensitive.

II. For the next card the pigment selected was geranium-red, which differs from vermilion by the addition of blue and violet, these colours being distinct to Lieut.-Col. Scott. This was found to match with a sort of French grey or slate-colour with no tendency to green. On the grey half of the card I painted GO in blue and NOT in geranium. The first word he could see at a glance, but the second was invisible to him, although to me it was more conspicuous than the other.

III. For the third card we took emerald green, mixed with a little yellow, to cut off some of the blue rays. This was found to match with a rather rich yellow ochre, made with Mars yellow, Oxford ochre, and a little burnt sienna. The letters P.T.O. were painted in the yellow ochre mixture on the green, and R.S.V.P. in emerald green upon the yellow ochre background.

He could not see either, the whole card appearing one uniform green to him. But he immediately detected the letters O.K. upon the green and A.B.C. and Z upon the yellow ochre in a salmon-pink made by adding a very little geranium-red to the yellow ochre, instead of the burnt sienna.

IV. For a fourth card we made a full-toned lilac with mauve, magenta and zinc white. The colour by which this was matched was a fairly pure blue quite free from any tint of purple, but showing in the spectroscope a good deal of green. On the lilac half I painted P.K.W. in blue, and on the blue half the letters X and I in a slightly paler blue, made by mixing a very little more zinc white with the ground colour. The difference is so slight that very few people can trace these letters through the perforated zinc, although they see the letters P.K.W. immediately.

Lieut.-Col. Scott, on the other hand, could not detect P.K.W. and the whole card appeared of one uniform bright blue tint to him, but he instantly perceived the letters X and I. I have noticed this same sensitiveness to depth of colour in other cases of red-blindness.

Complete or nearly complete red-blindness is immediately detected by these cards. Partial cases can sometimes read the letters, but do so with difficulty. Green-blind people easily read the first three, but are stopped by the fourth, being unable to distinguish between lilac and the greenish blue.

The apparatus has been in use in Oxford for colour-testing ever since we made it, and answers admirably. We found that the X and I on No. IV were easily visible to all the cases of red-blindness, but not to the green-blind nor the normal.

With regard to the permanence of the colours, we found in 1908, *i.e.* after five years, some slight changes had taken place in all but No. I, which had not altered after being oiled. In No. II the geranium had lost something of its blue and violet components, and in No. III the yellow mixed with the emerald green had faded, so that Lieut.-Col. Scott, who knew what to look for, could just make out the lettering. But other colour-blind people were still deceived and No. IV was as effective as ever with both the red- and the green-blind.

All test-cards, especially such as Nagel's and Stilling's, are liable to deteriorate, but the use of the perforated zinc enables fresh designs to be prepared with so little trouble that this objection is easily remedied in our test-cards. It is much to be desired that some one who is green-blind, or has otherwise abnormal colour-sensation, would prepare a set of cards with as much care and accuracy as Lieut.-Col. Scott did these for red-blindness.

Most of the testing was done in the Physiological Laboratory, Oxford, some in University College, Reading, and some were cases from the Oxford Eye Hospital. The expenses, which were trifling, were defrayed out of the Government Grant Fund.

The Locomotor Function of the Lantern in Echinus, with Observations on other Allied Lantern Activities.

By J. F. Gemmill, M.A., M.D., D.Sc., F.Z.S., Lecturer in Embryology, Glasgow University, and in Zoology, Training College, Glasgow.

(Communicated by Dr. E. W. MacBride, F.R.S. Received January 17,—Read February 29, 1912.)

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I. *Out of Water.*

Thirty years ago, in a paper to the Royal Society of London,* Romanes and Ewart pointed out that a sea-urchin uses its lantern in progression out of water, and gave an account of the manner in which the lantern acts. This account, however, seems to have been passed over completely, since there is no mention of it in the later book of Romanes, 'Jellyfish, Starfish, and Sea-urchins,' London, 1885, nor is it referred to in any of the general or special works dealing with Echinoderms that I have come across.† What one gathers from these sources, when they refer to the point at all, is that, out of water, sea-urchins are able to travel for short distances by using their spines as so many stilts, with the help of which they can pole their way along slowly and with difficulty, the motive power coming from the muscular collars at the bases of the spines. The writer has to confess that he was entirely ignorant of the account given by Romanes and Ewart in the paper above referred to, until after the whole of the following observations on locomotion had been made.

In view of the circumstance detailed above, and also as I have been able to obtain records of the action by a number of methods and to add much collateral information, it may not be amiss if a full and independent description be here given, all the more since that of the two authors named requires to be supplemented in one important particular (p. 91), as well as to be extended under certain conditions to locomotion under water.

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* "Observations on the Locomotor System of Echinodermata," 'Phil. Trans.,' London, 1881, pt. 3, pp. 829—855.

† *E.g.*, Bronn's 'Klassen u. Ordnungen,' Leipzig, 1904, vol. 2, p. 4; 'Cambridge Natural History,' vol. 1; 'Treatise of Zoology,' ed. by E. Ray Lankester, London, 1900, pt. 3; Delage et Hérouard's 'Traité de Zoologie Concrète,' Paris. 1903, vol. 3; L.M.B.C. Memoir on Echinus, by H. C. Chadwick, Liverpool, 1900.

In 1910, Miss Abel, a student attending one of the Nature Study Classes at the Millport Marine Station, noticed that the specimen I had set her to watch seemed to move by lurches occurring at intervals, and that just prior to each lurch the whole urchin rose up slightly from the table surface. I have pleasure in acknowledging this observation as the starting point of the following paper.

The fact was verified in Miss Abel's specimen as well as in several others, and it was seen that the rising, prior to each lurch, was effected by protrusion of the lantern of Aristotle, the tips of the five teeth being brought together at a certain stage in the movement, and forming a powerful central stilt on which the greater part of the weight of the urchin for the time being rested. It was surmised that, besides lifting up the urchin, the muscular apparatus of the lantern also pushed it forward at each lurch, the place where the teeth rested on the table serving as the fulcrum or fixed point in the movement. A record of the direction and extent of the movements of the first urchin was then made by setting it to travel over a large sheet of paper, and making a dot on the paper immediately below a marked spine, at the end of every lurch. This record is reproduced in fig. 1. Nothing further could be done at the time, but during last spring and summer I had the opportunity of examining the question further with the help of the plentiful material available from the tanks at the Millport Station. It is perhaps an indication of the healthy character of these tanks that urchins which had been in them for three weeks or so, travelled just as vigorously as others which had been brought in on the actual day of the experiment. The observations have reference primarily to *Echinus esculentus* L., but apply also in their essentials to *Echinus miliaris* (Gmel.), allowance being made for differences in size, length of spines, etc.

Description of Action.—When an urchin is travelling freely (p. 93) out of water, there occurs a rhythmic swinging movement of the lantern in the direction of progression. The movement is made up of a backward* and a forward stroke. The former is the pushing or poling stroke and is accompanied by protrusion. The latter is the return stroke, serving to bring the teeth forward again into position, and it is naturally accompanied by lifting or retraction of the lantern. In detail, the sequence of events is as follows, for urchins of small or medium size (*e.g.* up to $3\frac{3}{4}$ inches in equatorial shell diameter):—Just after a lurch has been accomplished the lantern points downwards and backwards, and the tips of the teeth, which are firmly closed and strongly protruded, lie a little behind the centre of gravity of the whole urchin, the posterior edge of which is slightly tilted up. The lantern is next retracted so that the teeth are lifted from contact with the supporting surface. The tilting up of the posterior edge now disappears, and the lantern is then moved pendulum-wise till it comes to point downwards and forwards, and the tips of the teeth are slightly in advance of the centre of gravity of the whole urchin. This is what we have called the return swing. Early in it, the tips of the teeth begin to diverge slowly from one another. The separation increases up till the end of the swing, and reaches its maximum immediately after the commencement of the second part of the whole movement, *i.e.* the part which we have called the pushing, or poling stroke. This part is initiated by protrusion downwards and forwards of the very widely separated tips of the teeth. The latter begin to

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* The terms *forward*, *backward*, *anterior*, *posterior*, *lateral*, have reference here to the direction of progression.

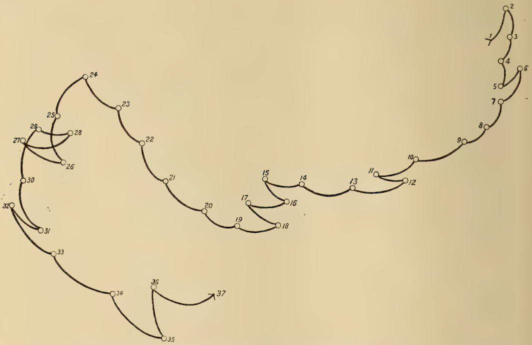

Fig. 1.—Record of track ($\times \frac{1}{2}$) obtained by setting urchin on a large sheet of paper, marking a particular spine, and placing a small circle on the paper below this spine at the end of each lurch. The lines connecting the various dots indicate approximately the path taken by the spine. The total distance travelled by the central part of the urchin amounted to about 14 inches, the number of lurches was 37, and the urchin itself measured about $3\frac{1}{2}$ inches in equatorial shell diameter. The track is less regular than the usual type. (Drawing made by Miss Abel.)

come together, but are still more than half open when they first strike against the supporting surface. Protrusion now continues, so that perforce the whole urchin begins to be slightly lifted up. Since at the commencement of the pushing stroke the tips of the teeth are in advance of the centre of gravity of the urchin, one finds that the lifting up chiefly affects the anterior half of the shell, which accordingly becomes distinctly tilted. Meantime the tips of the teeth have come firmly together and are pressing hard against the supporting surface. The whole urchin is now pushed slowly forwards, partly by the action of the spines, but chiefly by that of the lantern itself, through its muscles.

The lantern and its muscles act like a lever of the third order, the tips of the teeth being at the fulcrum, and the weight being centred somewhere inside the shell. In this manner the weight is gradually moved till it comes to lie in front of the fulcrum, and then the urchin tumbles forward under the influence of gravity. The anterior edge is no longer tilted up, but rather becomes depressed as compared with the posterior one, which in its turn is propped up for a time by the teeth. The latter are still protruded strongly, but now point backwards, and, by pushing to their furthest limit against the supporting surface, increase the distance travelled by the urchin in its forward lurch.

It is, however, only when the urchins have, so to speak, got "into their stride" that one sees matters proceeding exactly in the above manner. At first, when the steps are shorter and the direction less definite, the alternate tilting up of the anterior and posterior edges is not well marked. Gravity accordingly plays a smaller part in the lurches, each of which is rather a push forward over the tips of the spines than a rapid stumble or lurch. This applies at all times to the movements of the larger sized urchins, *i.e.* those in which the equatorial shell diameter is greater than 4 inches. The lantern is here smaller in proportion to the size of the whole shell, and the amplitude of forward or backward swing of which it is capable is not great enough to bring the tips of the teeth very sensibly in front of, or behind, the centre of gravity and thus produce the tilting above referred to. Plasticene records and other methods of demonstration show, however, that the lantern is still used in exactly the same manner as before, and that the "steps" are still definite, though each is accomplished more slowly and tends to exhibit minor hitches, which depend on the varying amounts of forward movement possible over the particular spines on which the urchin is from time to time supported.

Muscles involved.—On the whole, the anterior* and posterior protractor and retractor muscles may be described as acting in *converse* agreement, in order to produce the swinging part of the movements. That is to say, the anterior protractors and the posterior retractors are associated together alike in relaxation and contraction, while there is a similar harmony in the case of the anterior retractors and the posterior protractors. However, during the powerful downward push of the lantern, which lifts up the urchin, the whole set of protractors must act together. Later, during the backward push of the lantern, the posterior retractors will be in action, while, conversely, the posterior protractors are more or less relaxed. When it is remembered that the protractors tend to close the teeth and the retractors to open them, an explanation will readily suggest itself of the fact that, towards the end of the backward push, the lateral teeth are often seen to be more closely pressed together than the anterior and posterior ones. This point is brought out in fig. 2, and is explained in the notice attached.

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* See note on p. 85.

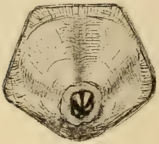

Fig. 2.

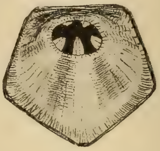

Fig. 3.

Fig. 2.—Sketch of the teeth and oral membrane towards the very end of the phase of protrusion. As in fig. 3, the direction of the urchin's movements was towards the top of the page. The teeth are strongly protruded and point backwards, their tips being brought closely together. The lateral teeth are here shown as coming together more closely than those in front and behind. This condition, like the converse one illustrated in the previous figure, is often seen, and is due to the fact that the posterior retractor muscles alone of the retractor set are now in action, in order to slant the lantern in such a way that the necessary *backward* push will be given to the tips of the teeth acting against the supporting surface. Meanwhile all of the protractors, except the posterior ones, are in action. It will be remembered that the protractor muscles serve to close the teeth.

Fig. 3.—Sketch of the teeth and oral membrane as they appear a little after the commencement of the phase of protrusion. The direction of the urchin's movement was towards the top of the page. At this time the œsophageal papillæ (not shown in the sketch) are swollen out so as to fill in great part the space between the teeth. The posterior tooth is here shown as being slightly less divergent than the others. This condition is often seen at the particular stage illustrated. It is due to the fact that, in order to permit the forward swing of the teeth which accompanies retraction, the posterior retractor muscle must have been in a state of at least partial relaxation. It will be remembered that the retractor muscles serve to separate the teeth.

Retraction during the forward swing will be effected chiefly by the joint action of the four anterior pairs of retractor muscles. The retractor muscles tend to pull apart the tips of the teeth, and this circumstance accounts for the separation of the teeth occurring during the return swing, and also for a feature frequently noted, that the posterior tooth, or pair of teeth, is now less divergent than the others, since the posterior retractor muscles are in a condition of, at least partial, relaxation in order to permit the advance of the tips of the teeth.

Recording Surfaces.—The best means I could hit upon for getting an accurate record of the length of each step was to set the urchin to travel over a thin, smooth layer of plasticene, spread on a suitable backing, *e.g.* wood, glass, or stout paper. Records are quite easy to obtain by this method, and a photograph (natural size) of one such record is given in fig. 4. It was made by an urchin of $3\frac{3}{4}$ inches equatorial shell diameter, and shows not only the marks of the teeth, but also those of the spines. The best kind of surfaces for urchins to travel over are those which are hard enough to resist the protrusion of the teeth, and at the same time not so smooth as to make the teeth liable to slip during the push. Glass is accordingly not one of the best;

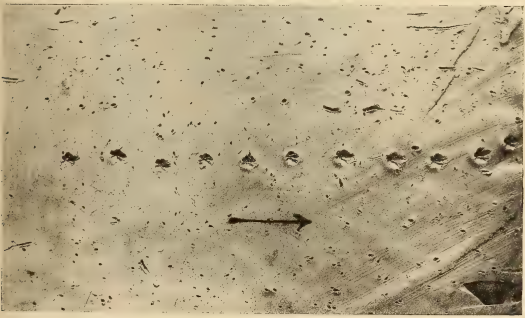

Fig. 4.—Photograph (natural size) of track left on plasticene out of water by an urchin of $3\frac{3}{4}$ inches equatorial shell diameter. The direction of movement was from left to right along the track. The pushing action of the teeth is indicated by the slope of the tooth-marks, particularly in the later steps, where the plasticene happened to be more thickly spread. The time occupied was 7 mins.

still, it gives sufficient purchase to enable urchins of large size to progress by short steps, while urchins of the smaller and medium sizes can travel over it almost as quickly as over wood or plasticene. At the same time, while the longest steps I observed on glass measured about $\frac{3}{8}$ inch, the records on plasticene showed some steps which rather exceeded $\frac{1}{2}$ inch. A certain amount of slipping must accordingly have taken place on the glass, and this is borne out by the facts which are noted at the end of next paragraph and also on p. 97.

Strength of Effort.—Not only are the lantern muscles strong enough to raise and push the urchin intermittently in the manner previously described, but they are able to effect this, even although the shell be loaded with a considerable weight. Progression by short lurches can still take place in specimens of 3 inches diameter under loads of half a pound and under, while larger urchins are just able to move along for short distances carrying as much as 15 or 16 ozs. Under these circumstances the impressions left by the teeth on surfaces prepared to receive them are deep and decided, the length of each step is short, and movement is impossible over smooth hard surfaces, on which the teeth and spines can obtain no purchase.

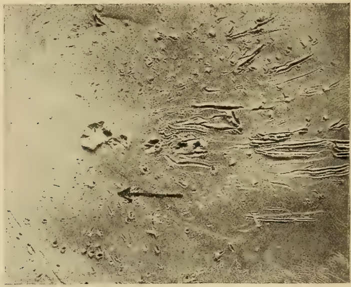

Fig. 5.—Photograph (natural size) of track left on plasticene by a loaded urchin travelling out of water (p. 90). Movement was from right to left. The heavy dragging of the spines will be noted. Five steps were taken, and then the urchin could advance no further, but was only able to bite at the plasticene, making a large pit, at the bottom of which is seen the white cardboard backing on which the plasticene was spread.

Climbing.—An urchin of small or medium size travelling actively with the help of the lantern can surmount considerable inequalities of surface. For example, it may be guided by stimulation to hitch itself for some distance up a stair, the successive steps of which, if broad enough, may be as much as a fifth of an inch in height. Such steps would prove an insuperable obstacle were it not for the powerful aid afforded by the lantern. Compare climbing uphill on a plasticene surface under water (p. 97).

Marks of Teeth on Laminaria.—Urchins of the medium and larger sizes (from $2\frac{3}{4}$ inches in diameter upwards) leave the mark of their teeth when travelling over a frond of Laminaria. Each mark exhibits five dents, some little distance apart, showing (what is brought out also by the various other methods of observation) that the teeth are not closed at the time when they first impinge against the supporting surface. The surface of the frond is not, however, broken through at these dents, still less is any part actually bitten out. The smaller urchins have not weight enough, unless when artificially loaded, to leave the marks of their teeth on Laminaria, but down to the smallest size investigated ($\frac{7}{8}$ inch diameter) they will leave such marks, even when unloaded, if made to travel over a surface of plasticene.

In the paper already referred to (p. 84) Romanes and Ewart ascribe only a lifting action to the lantern, the push being apparently given by the spines alone. I am convinced that the lantern also pushes strongly, indeed much more strongly than the spines, in active travelling. Observation by means of a simple arrangement of mirrors allows one to watch the action at leisure. The dents and scratches made by the teeth on plasticene (fig. 4) or paraffin wax always tend to take a backward slope. Lastly and most convincingly, small and medium-sized urchins, the oral surface of which has been denuded of spines, can move by almost as large and as quickly repeated lurches as other urchins of corresponding size in which the spines are intact. The observations on urchins travelling under water (p. 97) have also a bearing on this point.

Progression by Spines only.—It has just been stated that urchins of small and medium size may travel freely by the action of the lantern alone after all the spines on the oral surface have been removed. Can the spines alone enable the urchin to travel? The difficulty here is to exclude the use of the lantern without causing such injury as would interfere with co-ordination of the spines. One simple method consists in placing the urchin mouth downwards over a hole in a piece of wood, the hole being just so big that its edges are clear of the tips of the teeth when swinging begins. Too big a hole puts the spines near the mouth out of use, but if the direction of the lantern swing be first determined by irritation, a long narrow hole can be used. The general result of this line of experiment and of the plasticene records is to show that urchins travel very slowly, and never further than an inch or two, if their lanterns are not in use.

Urchins not infrequently cast off all but the smallest of their spines, and renewal and re-growth to normal size are probably slow. At any rate, among equal-sized urchins, one finds extreme differences as regards the number and size of the spines. There are, however, no corresponding differences in the rates at which the urchins travel out of water. Indeed, the average results, as worked out from a large number of instances in which the relative lengths of the spines were noted, showed that the advantage, as regards speed and distance travelled, lay rather with the short and medium-spined urchins than with the long-spined ones, in cases where the lantern of Aristotle had been in use.

Rhythm.—The time required for the various movements involved in a single lurch is fairly uniform for the same urchin if travelling undisturbed, but shows some irregularity when changes of direction are adopted. The rate of swing is only slightly accelerated by stimulation, but becomes retarded as vitality is lost. The average frequency varies slightly among healthy specimens of similar size, but shows marked variation according to differences in size, the smaller urchins having higher frequencies. The following data may be quoted from examples actually observed :—

Equatorial shell-diameter of urchin.	Time in seconds between six successive "steps."	Average length of "step."
in.		
1	15, 16, 14, 15, 15, 16	$\frac{2}{16}$ of an inch.
$1\frac{1}{2}$	20, 23, 22, 22, 21, 23	$\frac{2}{16}$—$\frac{3}{16}$,,
$2\frac{1}{2}$	25, 27, 18, 18, 26, 27	$\frac{3}{16}$—$\frac{5}{16}$,,
3—$3\frac{1}{2}$	36, 37, 50, 50, 36, 36	$\frac{5}{16}$—$\frac{8}{16}$,,
4	45, 44, 46, 46, 44, 45	$\frac{6}{16}$—$\frac{8}{16}$,,
5	50, 52, 65, 44, 51, 49	

Length of "Step."—As indicated in the last column the length of step varies according to size, the smaller urchins naturally taking the shorter steps. At the same time, after an equatorial shell diameter of $3\frac{1}{2}$ inches has been reached, there is practically no further increase in the length of the steps. Urchins of $2\frac{1}{2}$—$3\frac{1}{2}$ inches proved the best for travelling. The larger urchins are more sluggish than those of medium size, and their weight is probably greater in proportion to the size of the lantern and the strength of its muscles.

Change of Direction.—It is well known that urchins will change their line of progression to a direction away from a part irritated. Out of water, a considerable time elapses before the change becomes obvious through actual movement of the whole shell. Much earlier, however, as careful watching shows, the lantern of Aristotle has begun to change the direction of its rhythmic swing, and a large part of the delay is due to the fact that the teeth cannot at once get themselves into proper position for the commencement of a lurch. One or more ineffectual swings have usually to occur before the first step is actually taken. These swings tend to be intermediate between the old and the new directions, and work round gradually to the latter (see fig. 8).

Inversion.—As was pointed out by Romanes and Ewart, if an urchin be turned upside down out of water, the lantern will exhibit rhythmic swinging movements accompanied by protrusion, retraction, closing and opening of the teeth. These movements are just those of the locomotor swing. In vigorous specimens they are even slightly greater in extent, as well as quicker in rhythm, than when actually employed for movement. A very interesting point is that the direction of swing of the lantern in an inverted urchin can be made to change by stimulation, and that the new direction adopted is always the one that would lead the urchin away from the stimulus were it free to travel. As noted for the normal position, in the previous paragraph, the change of swing of the lantern takes some little time to manifest itself, and is usually initiated by one or more short intermediate oscillations.

Equatorial Section.—If an urchin shell be divided equatorially, and the lower half, still full of cœlomic fluid, be set mouth downwards on a suitable surface, it will still in some instances progress, though never freely or far, both when free of load and when carrying moderate loads. Under these conditions it is possible to observe from the inside the swinging of the lantern during the course of each lurch, and I have obtained records on plasticene demonstrating the corresponding action of the teeth. At best, however, no doubt owing to the shock caused by the division of the shell, the movements are very far from being regular or persistent.

General Facts regarding Progression.—If a number of apparently healthy urchins be taken at random out of the tanks and placed mouth downwards on a table, about half of them will begin to travel with considerable freedom, the rest remaining sluggish. It is hard to say what causes the difference in each case. Small and medium-sized urchins start off more readily than large ones, but the same urchin may go freely at one time, and at another hardly shift its place. In the latter event the lantern is either not in use at all, such movement as occurs being caused by the spines, or more rarely the teeth are only knocking or biting up and down against the surface. In either case irritation, as by scratching one side with a needle, is sometimes, but not always, an effective starter. It would seem as if a certain amount of physiological inertia had first to be overcome before the nerve stimuli could be released on which co-ordinated movements of the lantern depend. Habit also counts for something. The most persuasive method of starting the movements is to set the urchin upside down and apply irritation to one side. Once the movements have begun, you may expect with confidence that the urchin will travel freely if it be restored to the mouth-downward position, even though prior to inversion it had refused to budge at all. Similarly, if it be next immersed in sea water so that it can travel by help of spines and sucker feet alone, it may continue to exhibit an ineffective, but still rhythmic and regular lantern activity. Inertia has been replaced by momentum.

Urchins which one can recognise as being in an unhealthy condition fail altogether to travel out of water, though under water they may still be able to move about by using their sucker feet.

Distance Travelled and Time Taken.—The furthest I have noted urchins to travel out of water is 12 to 16 inches in 20 minutes. Usually the distances are shorter, and, in over 100 experiments, just 45 per cent. of the urchins went upwards of 4 inches in the time named. The direction of progress cannot be predicted. The same urchins, similarly oriented, in successive experiments at intervals of an hour or so, often started off in quite different directions from those which they had previously adopted, although the greatest care was taken to prevent surface irritation during the experiments, as well as during the intervals when the urchins were replaced in the tanks. The lines of progression may be divided into three groups, as follows :—

(*a*) Straight, or showing only slight irregularities, usually wavy in character, from side to side.

(*b*) Curving more or less uniformly and quickly to one side.

(*c*) Showing one or more distinct changes of direction. The tracks intervening between the changes fall naturally under *a* or *b*.

The proportion of instances in which the above lines of progression were adopted in my experiments and the relation to rotation are shown in the table under the next heading (p. 96).

Rotation.—Out of water urchins usually exhibit a certain amount of rotation as well as progression. The two movements are associated together in the sense that both are caused by the action of the lantern and of the spines, but, so far as I can judge, they do not depend on one another in any intimate or causal sense. There may be rotation without progression, and progression without rotation, and when they occur together there is no fixed ratio between them. For example, an urchin may travel 12 inches and yet have practically the same orientation at the end as at the beginning, or it may rotate through two or three right angles without shifting its place for more than an inch or so. Again, if it does travel actively and rotate at the same time, the rotation may be very slight (10°) or may amount to a full turn of the circle. At the same time, rotation and progression probably affect one another in determining the character of most of the tracks which come under group (*b*) as well as of some which fall under group (*a*). Taking the latter first, we find that there are instances in which each forward step is accompanied by a slight twist to one side or the other, while in the next lurch there occurs a similar twist, but in the opposite direction. Or several successive twists may be to one side, and these may next be rectified by a (roughly) corresponding number in the opposite direction. The line of progress here shows wavy deviations from side to side, and at the end of the journey little or no rotation has been effected. Such instances, however, only form a small proportion of group (*a*). As will be seen from the accompanying table the average amount of rotation in this group is still distinctly high.

It is in the cases coming under group (*b*) that an association between rotation and progression is definitely indicated. This group is marked by continuous curving of the track to one side or the other. From the table it will be seen that in the great majority of instances rotation in *the same direction* also occurs.

The factors that produce rotation seem to be twofold. First, there occurs a screwing action transmitted through the lantern, and caused by unequal contraction on the part of the single muscles making up each pair of protractors and retractors, or by unequal contraction on the part of the protractors and retractors at opposite sides. When the teeth are opened and pressed hard against a surface, sufficient power and purchase is thus obtained to produce slight rotation of the whole urchin, and this movement may be aided by pushing on the part of the spines. No doubt the cases where there was rotation through two right angles or more, without sensible progression, are to be explained by successive small twists of this character. It is not improbable that the habit may be of use in feeding by enabling the urchin to dig in its teeth with greater effect, and to repeat the bites many times without travelling far away from ground which it may have found to be suitable. Possibly also the action is reminiscent of a former boring habit, or related to this habit in other urchins (p. 101). Should the action occur while the urchin is travelling, the track will tend to curve in the direction of rotation. There will obviously be no definite limit to the total amount of deflection and rotation thus produced.

The second element in the production of rotation has reference to the spines alone. These have always to play their part in travelling, chiefly as supports, but also to some extent as active pushing agents. Should they be defective or inactive or not straightened out over any part of the walking surface of the shell, that part will tend to lag behind at each lurch unless it happens to be already right behind or right in front. The lagging of one side will produce an apparent rotation of the shell towards that side, and will also cause the line of progression to be deflected in a corresponding direction. Here the amount of deflection and rotation cannot possibly exceed two right angles, and will usually be much less.

The very marked relation between progression and rotation shown in the tracks which fall under class (*b*) seems capable of satisfactory explanation on the above lines.

If one injures a group of spines on the walking surface, so much irritation seems to be caused that purely passive or mechanical factors are superseded. At least I found it quite unsafe to predict that a recently injured part would lag behind. The handicapping effect of the injury seems to be overridden by the screwing action of the lantern and spines, and this action in turn must depend on nerve reflexes, the analysis of which falls outside the scope of this paper.

Ratio of Rotation to Progression.—To give an idea of amount of rotation relative to progression, I adopted, in the case of each experiment, the ratio obtained by dividing the total rotation in degrees by the amount of progression in inches. The average value of this ratio in groups (*a*), (*b*) and (*c*) is given below, and it will be noted that the table only includes instances in which the amount of progression in 20 minutes was upwards of 4 inches. From what has been said before, it will be evident that, if all instances be taken into account, the R/P ratio may vary from nothing to 180 or more. In a single lurch there may be as much as 20° of rotation.

Table showing Direction of Progress and Relation to Rotation in 40 instances in which the Total Amount of Progression exceeded 4 inches (100 mm.) in 20 minutes.

(*a*) Progression along a line which is straight or shows only small variations from side to side; 14 instances	Without rotation, 2 instances. With rotation, 12 instances; average R/P ratio, 16. Dextral* rotation, 6 instances. Sinistral rotation, 6 instances.
(*b*) Progression along a line curving more or less uniformly towards one side; 23 instances; average R/P ratio, 11	Curvature of progression corresponds with direction of rotation, 18 instances. Both dextral, 10 instances. Both sinistral, 8 instances. Curvature of progression does not correspond with direction of rotation, 5 instances.
(*c*) Progression along a line showing one or more sudden changes in direction; 3 instances; average R/P ratio, 10½.	

* In the watch-hand direction as viewed aborally.

II. *Under Water.*

In ordinary circumstances the lantern of Aristotle is of little importance as regards locomotion under water. Even large urchins will travel mouth downwards over a smooth plasticene surface without leaving the marks of their teeth. The sucker feet are now in action and, in addition, the spines are able to play their part not merely as passive supports, but, by means of the muscular collars at their bases, as active agents in pushing the urchin along, now that its weight is so greatly lessened. Indeed, under water a vigorous urchin can move with no little freedom over a horizontal surface without using anything but its spines. Accordingly, although a rhythmic swing of the lantern in the direction of progression may still occur, the teeth are not protruded so far or so vigorously at any time as to press hard against the supporting surface.

Again, in ordinary movements on a vertical surface the sucker feet and spines seem to suffice. But when the urchin is travelling quickly upwards on such a surface it may use its lantern with powerful effect to aid the progress. If one observes this through the wall of a thin glass vessel, one can hear the grating of the teeth against the glass and see them slipping in the middle of the push, or even twice or thrice in each push, owing to the smoothness of the surface. I noted this particularly in some urchins brought into sea-water which had first been boiled, to expel the air, and then cooled down to ordinary tank temperature (p. 106). No doubt, in nature, the lantern will be of no little use in quick upward progression on any vertical surface which is sufficiently rough to allow the teeth to push without slipping, and yet smooth enough to give attachment to the sucker feet.

However, even on a horizontal, or practically horizontal, surface, I have been able by three methods to compel the use of the lantern for locomotion under water, and by two of these methods to obtain a record on plasticene of the marks left by the teeth, while in using the third I had to be content with ocular observation of the action. Of the first two methods, one consisted simply in loading the urchin with a weight of half a pound or more and setting it to travel on a horizontal plasticene surface (fig. 6). In the second method I left the urchins unloaded, but induced them to travel up an inclined plasticene surface by first setting them on such a surface and then irritating the downward side. The sucker feet are able to come into action against a smooth plasticene surface, but not so effectively as, for example, against glass, and this circumstance, combined with the fact that the weight of the shell had to be lifted up the slope, apparently made it necessary that the action of the feet and the spines should be reinforced by that of the

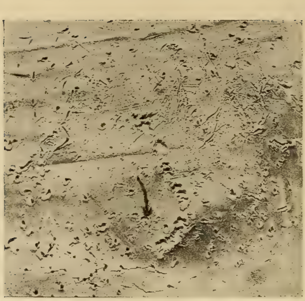

Fig. 6.—Photograph (natural size) of track left by a small urchin heavily loaded and travelling under water on a horizontal surface covered with a thin layer of plasticene. Numerous very short steps were taken. The slanting of the dents indicates the direction of movement, *viz.*, towards the foot of the page.

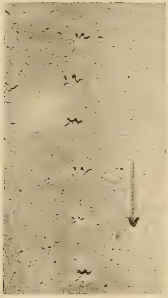

Fig. 7.—Photograph (natural size) of track left by a larger urchin completely immersed under water and induced to travel up an inclined plane of smooth plasticene in the manner described. The marks of the teeth are slanting but not deep. The direction of movement was towards the foot of the page. No mark at all has been left by the anterior tooth, *i.e.*, the tooth on the side towards which the urchin is travelling.

lantern (fig. 7). Too gentle a slope will not give the desired result, and in my experiments it was only with an incline of 25° or over that a record could be obtained.

The third method consisted in setting the urchins to travel over a tightly stretched surface of fine tow-netting silk placed in a glass vessel, the bottom of which was thin and smooth enough to allow observation through it. One then watched what happened, by means of a simple arrangement of reflecting mirrors. The meshes of the silk interfere with the action of the suckers at the ends of the podia, and the movements of the spines are, to some extent, hindered through the tips sticking in the meshes, while at the same time the silk is sufficiently transparent to allow the teeth to be distinctly seen when they are protruded and brought up hard against it. Under these circumstances, both when loaded and unloaded, urchins may be observed to use the lantern freely and with effect.

Partial Immersion.—Urchins that are only partially immersed under water will be found to use their lanterns for locomotion even over horizontal surfaces, in a degree varying inversely with the depth to which they are immersed. One may evoke stronger or weaker efforts on the part of the lantern simply by siphoning water out of, or into, the vessel in which the urchins are contained. Fig. 8 shows a photograph of the track left on plasticene by an urchin which was just a little more than half immersed (see explanation appended to the figure).

The employment of the lantern, as described above, may at times be of the greatest service to urchins in the lower tidal zones, by enabling them to escape back into deeper water should the outflowing tide threaten to leave them in an unfavourable position.

Under water, unless when heavily loaded, even large urchins will leave no impressions of their teeth when travelling over a frond of Laminaria. The bearing of this on the question of feeding is referred to later (p. 101).

The lower half of an urchin placed mouth downwards may travel for short distances under water, but, like the uninjured urchin, it will not, except when loaded, leave impressions of its teeth on a plasticene surface.

In nature, urchins may have to travel under water over horizontal surfaces (*e.g.*, sand, mud, rocks, etc., covered by growths of fine algæ, etc., or powdered with sand or mud), where the sucker feet cannot come into action, and where it may be of great advantage that the action of the spines should be reinforced by that of the lantern. This is the more important since there is evidence that urchins (*Echinus esculentus*), whether normally or pathologically, not infrequently shed all but the smallest of their spines, and as spines are structures of somewhat slow growth, were it not for the lantern such urchins would be comparatively helpless in surroundings where their sucker feet could not take hold.

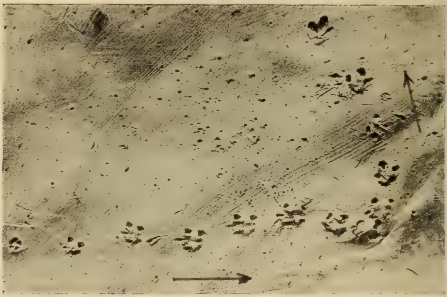

FIG. 8.—Photograph (natural size) of track left by an urchin ($3\frac{3}{4}$ inches in equatorial shell diameter) which was travelling over a plasticene surface while only rather more than half covered by sea-water. All the sucker feet, which are normally used when a completely immersed urchin is travelling mouth downwards, were in action in this instance, but nearly half of the bulk of the urchin did not have its weight neutralised by submergence. Accordingly, the lantern has been used with effect, and yet has not needed to be protruded in each step with sufficient force to bring the teeth firmly together. Separate dents are thus left by each of the five teeth in each step. It will be remembered that powerful action of the protractors causes closure of the teeth (p. 88). The direction of the movement was from left to right. When the urchin reached the lower right-hand corner it was irritated on the side looking towards this corner. The crowding of the tooth-marks at this part is a record of those short and comparatively ineffective swings of the lantern which usually initiate a change of direction due to irritation (p. 93).

III. *Relation to Other Activities.*

(*a*) *Feeding.*—No doubt this is by far the most important function of the lantern. The movements employed will resemble in many respects those which have just been described, the chief difference being that little or no lateral swing will be required. However, in feeding, as in locomotion, there must occur:—(1) Commencing protrusion with teeth fully opened; (2) gradual closure of teeth, accompanied by fuller protrusion, giving firm pressure against the surface which is being bitten or cropped; (3) gradual retraction and swing into new position, accompanied by partial opening of the teeth. In (2) the weight of the urchin will not be sufficient to supply counter resistance. Even out of water the weight alone could only cause the teeth to make slight marks on a frond of Laminaria (p. 91). Under water there must be strong fixation by the sucker feet, and the need for fixation will be all the greater when the urchin is grazing on a vertical surface. As a matter of fact, urchins travelling out of water over the prepared plasticene surfaces often took a small bite out of the plasticene at each step. The boluses, however, were not properly taken up into the gullet, but tended to remain within the grasp of the lower ends of the teeth.

The screwing action of the lantern referred to on p. 95 will obviously be of use in biting into hard or tough surfaces as well as in separating weeds, barnacles, worm tubes, etc., from the surfaces to which they are attached.

Boring.—In the Clyde area, *Echinus esculentus* and *Echinus miliaris* are not recognised as having a boring habit, though F. Caillaud* ascribes such a habit to the latter on the coasts of Brittany.

The same author states that *Strongylocentrotus lividus* and *Echinus miliaris* excavate cavities by a knocking or striking action of the teeth. P. Fischer† agrees in general with Caillaud, while M. Hesse‡ also records *Echinus miliaris* as having a boring habit. G. John§ gives a full description of the cavities made by different urchins in the lava rocks of San Miguel, and believes that these cavities are formed chiefly by the action of the teeth, though the spines also play a part, probably by giving the urchin a rotary movement.

If into the knocking action of the teeth spoken of by Caillaud we read just such movements as I have shown to occur in locomotion, viz.: protrusion of the teeth in the open condition, followed by still further protrusion with powerful closure and a sidelong push, we shall get an action which, in course of time, may well excavate cavities in rocks of moderate hardness. The teeth will play the chief part, as is natural considering the extreme hardness of their tips and the manner in which their length can be maintained by growth from the permanent tooth germs. It is noteworthy that certain urchins with boring habits elsewhere do not exercise these, where there are no tides, as in the Mediterranean.*

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* "Observations sur les oursins perforants de Bretagne," 'Rev. et Mag. de Zool.,' 1856, sér. 2, vol. 8, pp. 158, 179; "Supplément à des précédentes observations," 'Compt. Rend.,' 1857, vol. 45, pp. 474—476.

† "Note sur les perforations de l'Echinus lividus Lamk.," 'Ann. Sc. Nat.,' 1864, sér. 5, Zool., vol. 1, pp. 321—332.

‡ "Note sur les motifs que déterminent les oursins à se creuser dans les rochers des réduits dans lesquels ils se logent," 'Ann. Sc. Nat.,' 1867, sér. 5, Zool., vol. 7, pp. 257—263.

§ "Ueber bohrende Seeigel," 'Arch. f. Naturgesch.,' 1889, vol. 55, pp. 268—302.

Gill Respiration.—In the tanks, urchins that are apparently healthy sometimes have their gills prominent and well filled, while an hour or two later the gills may be by no means conspicuous. Changes of this kind do not seem to be rhythmic in character. Probably they depend on such factors as variations in the total quantity of fluid within the lantern cœlom. A necessary condition is relaxation of the muscular tissue of the gill-walls (see below, p. 103). The oral membrane also will often be found to be flaccid when the gills are largest. But, so far as this paper is concerned, the important question as regards respiration is whether the rhythmic movements of the lantern have any influence in causing flux and reflux of the fluid contained in the gills. This question arises alike in connection with the powerful lantern movements previously described, and with the minor ones now to be noted. Under water, urchins which are otherwise motionless may for hours at a time show slow rhythmic movements of the lantern. These movements correspond with the locomotor ones if one supposes the latter to be greatly reduced. There is slight protrusion and retraction, with opening and closing of the teeth accompanied by lateral swing. The rate corresponds exactly with that in actual locomotion. I have had the opportunity repeatedly of watching the movements in question in specimens which had been left undisturbed for several days in glass-sided tanks provided with a somewhat feeble circulation. On one occasion at 11 A.M. the positions of several urchins showing the movements were carefully marked, and at 3 P.M. it was found that the urchins had not shifted their position, though the movements of the lantern were still going on.

Careful watching through the glass failed to reveal any corresponding rhythmic swelling or contraction of the gills. The same thing holds good regarding the major rhythmic movements of the lantern which subserve locomotion. There is, under ordinary circumstances, no obvious increase or diminution of the size of the gills during protrusion and retraction of the teeth. If such variations do occur they are too slight to be distinguishable amid the secondary movements imparted to individual portions of the gills by the spines, tube feet, and pedicellariæ which are in constant activity all around.

Ordinary Respiration.—In considering how renewal of the cœlomic fluid inside the gills of Echinus is effected one must not forget two facts, (1) the ciliation of the internal lining of the lantern cœlom, of which the gill cavity is a portion, and (2) the contractile nature of the gill wall.

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* Fischer, *loc. cit.*, and de Serres, Marc, "Sur l'action perforante de l'Echinus lividus," 'Compt. Rend.,' 1857, vol. 44, p. 72; and "Note sur l'Echinus lividus de l'Océan, considéré comme une espèce perforante," *ibid.*, 1856, vol. 43, pp. 405—6.

(1) If a single drop of methylene blue solution be injected by a needle which just penetrates through the mouth membrane into the lantern cœlom, parts of the neighbouring gill will show some coloration after an interval of a few seconds. Here we have a means which in itself seems almost sufficient for ordinary needs.

(2) In *Echinus esculentus* the gill-walls possess contractility. Uexküll* states definitely that in *Sphærechinus granularis* the gill-walls are destitute of muscular elements, and accordingly are entirely dependent for their movements on the pressure within the lantern cœlom. Obviously the question is of very great importance as regards the mechanics of respiration. In *Echinus esculentus* one may bring forward the following evidence for contractility:—

(*a*) Direct stimulation of a single gill, *e.g.* by touching with a needle, will cause retraction of this gill without the others being affected. In very young urchins, as Macbride† has shown, the lantern cœlom is divided into five separate compartments. However, in specimens measuring 2½ inches and upwards in equatorial shell diameter, I satisfied myself, by injecting methylene blue with all due gentleness, that these compartments communicate freely with one another.

(*b*) Under a low power of the microscope in the undisturbed condition individual terminal branches of any part of a gill may be seen occasionally to contract or expand, apart from emptying or filling of the rest of the gill. (This may be a local reflex depending on the condition as regards oxygen and carbonic acid of the contained cœlomic fluid.)

(*c*) Weak solutions of nicotine and certain other reagents will cause active shrinkage of the gills. Nicotine is well known as possessing an almost specific power of causing muscular tissue in urchins to contract strongly.

(*d*) Nitrite of amyl, on the other hand, has a relaxing action, and, if carefully employed, may be used to remove moderate nicotine contractions.

(*e*) The contractile property of the gill may be removed temporarily, but completely, by exposure out of water. This method is referred to further on p. 104. It is the best to employ in experiments regarding the influence of internal pressure on turgescence of the gills.

(*f*) Microscopic examination of sections of a gill shows just outside of the cœlomic lining a thin membrane with fine fibrils resembling those of Echinid muscle.

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* "Über die function der Poli'schen Blasen am Kauapparat d. regularen Seeigel," 'Mitth. Zool. Station, Neapol.,' 1897, vol. 12, pp. 463—476, taf. 21.

† "The Development of *Echinus esculentus*," 'Phil. Trans.,' 1903, vol. 195, pp. 285—326.

We may take it then as certain that, in *Echinus esculentus*, the gill walls are contractile.

Further, under normal circumstances, the gill-walls maintain a degree of tonicity sufficient to prevent them from responding readily to moderate rises of internal pressure. One may press on the teeth and oral membrane in a healthy urchin without affecting the gills. Less force similarly applied in the case of an urchin treated as under (*e*) causes the gills to swell out at once.

Under ordinary circumstances accordingly, in a healthy *Echinus esculentus*, it seems most probable that the gill circulation depends—(1) chiefly on the ciliation of the cœlomic lining; (2) in a minor degree on contraction and relaxation of the musculature of the gill-wall, occurring without rhythm and probably by reflex action; (3) also in a very minor degree on other slowly acting causes, *e.g.* variations in the total quantity of fluid within the lantern cœlom.

Forced Respiration.—What one might call "forced respiration" can, however, occur. This is evidenced by swelling and shrinkage of the gills, corresponding with that rhythmic functional activity of the lantern whose application to locomotion it was the first object of this paper to bring out.

Forced respiration of this kind can only take place effectively if the muscular walls of the gills be in a state of relaxation. Experimentally, exposure to air affords a good means of inducing this condition. The exposure should be done, as far as possible, without leakage of cœlomic fluid and consequent disturbance of internal pressure conditions. Keeping the urchin inverted for 10 or 15 minutes within a vessel with just enough sea-water to leave the gills uncovered will be found to give the result desired. Subsequent complete immersion, followed, if necessary, by mechanical stimulation, in order to induce or increase the rhythmic lantern movements, allows the effect of these movements on the now flaccid gills to be watched.

Shrinkage of the gills takes place during protrusion of the lantern. It is best marked towards the extreme of this phase and is followed by turgescence which, occurring with some suddenness at the commencement of retraction, persists throughout the retraction phase, and is even continued for a brief period into the commencement of the succeeding protrusion. The filling and emptying of the gills is quite regular, and affects all of them alike. The rationale of the process seems to be as follows:—The lantern and the oral membrane act as a diaphragm which rises and falls. With each descent of the diaphragm (*i.e.* during protrusion) the total space within the shell tends to be enlarged, so that some lowering of pressure is established. This lowering of pressure also affects the lantern cœlom, the walls of which in many parts are thin and highly mobile. Accordingly, the fluid within the gills flows back into the lantern cœlom. On the other hand, when the diaphragm rises (*i.e.* during retraction), the internal pressure is heightened throughout, and the gills are refilled by an outflow of fluid from the lantern cœlom. A corresponding tendency to slightly greater fullness of the tube feet and anal plate can also sometimes be observed. Normally the anus is kept closed. Uexküll (see below) noted that during retraction of the lantern in *Sphærechinus granularis*, when the anus happened to be open, a compensatory outflow of fluid from the last part of the intestine might take place, and suggested that in this way the evacuation of fæces might be aided. He also rightly pointed out that adjustment of internal to external pressure can take place by the passage of water through the mouth. But immediate compensatory flux and reflux can hardly be supposed to take place by the mouth when one remembers the usual condition as regards narrowness of the œsophagus, and the manner in which the œsophageal papillæ swell out towards the end of retraction and during the first stage of protrusion. This last circumstance, as Uexküll noted, is an indication that the internal pressure is greatest towards the end of retraction. It is quite possible, however, that the process of swallowing may be aided by the suction which protrusion of the lantern is capable of exercising, supposing the œsophageal canal to be open.

Paralysis of the gill musculature can also be obtained by rinsing the gill gently with an 8 per cent. solution of chloral hydrate in salt water, and then washing thoroughly with clean water after an interval of 10 or 15 seconds. This produces some slight rigidity of the gill wall along with the paralysis, so that for observational purposes the method is not so good as that of exposure to the air, which leaves the gill wall flaccid. It has, however, the advantage of causing cessation of movement in the surrounding spines, etc., if these have also been rinsed with the chloral.

If one pierces the shell and the buccal membrane by fine holes into which capillary tubes are inserted, one may sometimes observe in these tubes a slight rise and fall of the fluid level accompanying each retraction and protrusion of the lantern. This is best seen in urchins out of water. But it will be found that with fine tubes there is often failure of response to slight variation of pressure. A very sensitive method, which, however, can only be applied out of water, is to bring the capillary tubes into such a position that drops of escaping cœlomic fluid just begin to form at their outer ends. The rate of dropping is hastened during retraction of the lantern and lessened or reduced to zero during protrusion.

The question now arises, does the gill wall under physiological circumstances relax sufficiently to facilitate rhythmic filling and emptying? If water is thoroughly boiled to drive off all dissolved air and then cooled, and if urchins, especially those of small or moderate size, be brought into it, they will climb vigorously up to the surface, exhibiting in many cases rhythmic locomotor movements of the lantern and having the gills well expanded. Not in all, but in about a third of such instances, one can observe some degree of swelling and shrinkage of the gills accompanying retraction and protrusion of the teeth.

As regards "forced respiration," then, I have shown that in the rhythmic activity of the lantern we have a function capable of emptying and filling the gills when the muscular walls of the latter are relaxed, and that in one particular state of the surrounding water the required degree of relaxation may occur.

Uexküll's Experiments.—An interesting and suggestive set of experiments on the factors which control filling and emptying of the gills in the Mediterranean species, *Sphærechinus granularis*, has been made by J. von Uexküll (*loc. cit.*). He was able (by mechanical stimulation of the nerve ring in an opened urchin which had previously been immersed in sea-water saturated with CO_2) to induce rhythmic retraction and protrusion of the lantern. These movements were accompanied respectively by filling and emptying of the gills due to rise and fall of the pressure within the lantern cœlom. He states that rise of pressure is caused by compression of the cœlom and its sacculations, resulting from contraction of the mouth membrane, the depressor muscles of the radii, and the walls of the dental sacs. Lowering of pressure, on the other hand, appeared to be caused by contraction of the so-called compass sheet of muscle connecting the five forked radii. In single instances, he states that he was able to observe respiratory movements going on while the lantern as a whole remained at rest. Though he mentions the swinging of the lantern to one side or another, he does not recognise its ambulatory functions. He dismisses with less than due consideration the observation by Romanes and Ewart referred to above (p. 84), and states that in the normal medium—sea-water—an urchin never uses its lantern for progression. He does not ascribe to protrusion or retraction of the lantern, in themselves, any influence on the filling and emptying of the gills.

The chief difference between the kind of respiration induced by Uexküll in Sphærechinus, and the "forced respiration" of Echinus, is that, in the former, the lantern cœlom seems to act by itself as a pulsating chamber to the gills. In contrast I have to emphasise the fact that in Echinus simple protrusion and retraction of the lantern are associated with sufficient rise and fall of pressure within the whole internal cavity of the urchin to cause swelling and shrinkage of the gills when the walls of the latter are relaxed.

I have not yet had the opportunity of repeating Uexküll's experiments with due care on *Echinus esculentus*, but certain considerations make it very doubtful whether similar results would be got. To begin with, in the Mediterranean species, according to Uexküll, the gill-walls are flaccid, while the walls of the dental sacs are markedly contractile. In Echinus, on the contrary, the gill-walls have contractility and tonicity, while the dental sacs are very thin and are only weakly contractile. Lastly, to judge from Uexküll's figures, the sub-radial sacculations of the lantern cœlom (which are the portions of this cavity that could be most readily compressed or released from pressure) reach a greater size in Sphærechinus than in our Clyde species. On the whole, therefore, from the structural point of view, the lantern cœlom in Echinus does not seem well adapted to serve as an actively pulsating chamber for the rhythmic filling and emptying of the gills.

Effect on Water-vascular System.—The internal pressure being lessened with every powerful protrusion of the teeth, any elastic-walled internal cavity which communicates with the exterior will have a tendency to expand. Should the communication be valved against reflux, turgescence of the cavity will be produced or maintained. It seems to me that we have here a mechanism which may materially assist the ciliation of the stone canal, &c., in keeping up within the ampullæ that degree of fullness on which perfect action of the sucker feet must depend. The point is one which would be difficult to verify by experiment, but it is at least in harmony with the fact that no rigidly shelled Echinoderm without a lantern makes effective use of its podia in locomotion.

Summary.

I. *Locomotion out of Water.*—When travelling actively (p. 93) the urchin raises itself from time to time on the tips of its teeth in preparation for a forward "step" (p. 85). The step is then accomplished by means of (*a*) strong pushing or poling on the part of the lantern (p. 87); (*b*) similar but usually weaker action on the part of the spines (pp. 87, 91); (*c*) the influence of gravity acting at a certain stage (p. 87). After each "step" the lantern is retracted and swings forward, so that the teeth come into position for initiating a new "step" (p. 86). Plasticene surfaces may be used with advantage for recording the marks of teeth and spines (pp. 89, 90). The length of each step ranges from $\frac{1}{8}$ to $\frac{1}{2}$ inch, according to size and activity of specimen, and the time required from 15 to 55 seconds (pp. 92, 93). The longest distances travelled are from 12 to 16 inches in 20 minutes, but hardly

50 per cent. of the specimens went more than 4 inches in that time (p. 94). Active progression by the lantern alone, is possible in small and medium-sized urchins (p. 91). Progression by the spines alone is very limited indeed (p. 91). An urchin can travel with the help of its lantern even when loaded to the extent of half a pound or more (p. 90). There is usually some rotation as well as progression, but the two are not associated as cause and effect (p. 94). Rotation is caused by (*a*) screwing action on the part of the lantern and of the spines, and (*b*) defect, inactivity, or unfavourable position of a group of spines (p. 95). An analysis is given of the lines or curves of progression in relation to rotation (p. 96). Other points to which attention is directed are—Muscles involved (p. 87), strength of effort (p. 90), change of direction (p. 92), inversion (p. 93), equatorial section (p. 93), recording surfaces other than plasticene (p. 91), the inertia and momentum of the rhythmic action (p. 94).

II. *Locomotion under Water.*—Here the lantern is not needed for ordinary locomotion, particularly over more or less horizontal surfaces (p. 97). There are, however, various circumstances, normal and experimental, in which it is employed with effect, as, for example, when the urchins are loaded (p. 97), or travelling up a slope on certain surfaces (p. 98), or only partially immersed (p. 99), or mounting rapidly up a vertical surface (p. 97).

III. The locomotor action of the lantern in urchins is a particular manifestation of a rhythmic functional activity which can also subserve feeding (no doubt the chief function, p. 100), boring (p. 101), and "forced respiration" (p. 104). In addition, it possibly aids the swallowing of food (p. 105), the evacuation of fæces (Uexküll) (p. 105), and the maintenance of physiological turgescence in various internal cavities (p. 107). The gill-walls contain contractile elements (p. 103) which maintain tonicity (p. 104). Ordinary gill circulation depends chiefly on the ciliation of the cœlomic lining (p. 103). "Forced respiration" occurs in connection with the rhythmic lantern movements, when the muscular tonicity of the gill-walls is relaxed from any cause Filling of the gills then takes place during retraction of the lantern and emptying during protrusion (pp. 104, 106). Experiment shows that during protrusion there is a slight lowering of internal pressure within the shell, affecting both the lantern cœlom and the peri-visceral cœlom, while during retraction a slight corresponding rise occurs (p. 105). Uexküll's experiments on *Sphærechinus granularis* are noticed (p. 106).

The illustrations include photographs of tracks left on plasticene by urchins travelling actively out of water (fig. 4), partly immersed in water (fig. 8), completely immersed and going uphill (fig. 7), out of water and carrying a load (fig. 5), completely immersed and carrying a load (fig. 6).

My best thanks are due to Prof. E. W. MacBride for criticism and suggestions. I have also to express indebtedness to Mr. R. Elmhirst, the Superintendent of the Millport Marine Station, for much care in providing me with material.

Studies on Enzyme Action. XV.*—*Urease: a Selective Enzyme.*

By Henry E. Armstrong, F.R.S., and Edward Horton.

(Received January 26,—Read February 1, 1912.)

[*International Catalogue of Scientific Literature.*

D 1310, Q 8311 (Urea). Hydrolysis by urease (Soja bean).

D 1814, Q 1240 Urease (Soja bean). Action on urea.]

The production of urea from the metameric ammonic cyanate by Wöhler, in 1828, was naturally regarded as an achievement of great importance in view of the part the substance plays in the human economy—very nearly the whole of the nitrogen secreted being voided as urea; the synthesis of such a compound was a severe blow to the vitalistic conceptions which had been dominant in organic chemistry and justly excited attention, as it broke down the barriers up to that time held to be existent between the organic and inorganic world.

The attributes of urea are in many ways remarkable and scarcely in correspondence with the simple formula ordinarily assigned to it. It is generally spoken of in the text-books as *carbamide* but its properties are more nearly those of the isomeric *hydroxy-amino-imino*-derivative: an explanation may be found in the assumption that the two isodynamic substances are present in an aqueous solution in oscillatory equilibrium:—

$$\mathrm{CO}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}\right. \leftrightarrows \mathrm{C}\left\{\begin{matrix}\mathrm{NH}\\ \mathrm{NH_2}\\ \mathrm{OH}\end{matrix}\right. .$$

But it is probable that these two compounds, in turn, are in equilibrium with the hydrate common to both, $C(NH_2)_2(OH)_2$: as chemical changes are all reciprocal effects, the behaviour of urea is therefore subject to considerable variation and depends on the conditions under which it is placed; on this account, a name which is significant of the origin of the substance rather than of its functions is specially appropriate.

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* XIV, 'Annals of Botany,' 1911, vol. 25, pp. 507—519. I—XIII, these 'Proceedings,' Series B.

Equilibration of Urea and Ammonic Cyanate in Solution.—Though the conception that salts in solution must be pictured as in a state of constant flux was advanced by A. Williamson, in 1850, in his celebrated memoir on the Theory of Etherification, little notice was taken of the suggestion and the phenomena of reversible change were not taken seriously into account until Deville's work on dissociation at high temperatures attracted attention; since that time it has been realised gradually that the properties of solutions are such as to necessitate the general application of Williamson's contention.

Urea is a remarkably stable substance in solution and is but slowly hydrolysed even by heating it with acids and alkalies.[*] At ordinary temperatures no perceptible change takes place in the solution: that is to say, it remains neutral and does not contain an appreciable amount of ammonia; on this account, probably, the alteration it undergoes was overlooked until J. Walker and F. J. Hambly[†] drew attention to the fact that urea and ammonic cyanate are related reversibly, thus:—

$$\underset{\text{Urea.}}{CON_2H_4.} \quad \rightleftarrows \quad \underset{\text{Ammonic cyanate.}}{NH_4{\cdot}CNO.}$$

The conversion into the cyanate is easily demonstrated. No precipitate is noticeable on adding nitrate of silver to a cold solution of urea; if, however, the solution be heated to 80°—100°, then quickly cooled and mixed with the silver salt, cyanate of silver is at once thrown down; if equivalent quantities of the two substances are heated together, a very large proportion of urea is converted into cyanate, indeed the conversion may be rendered almost complete by concentrating the solution.

The silver precipitate contains only traces of carbonate when urea is used initially, but when ammonic cyanate is transformed into urea, a considerable proportion—as much as about 4 per cent.—is converted into carbonate.

When equilibrium is established, a solution of urea which has been heated at 80°—100° appears to contain at most about 5 per cent. of the metameric cyanate and at lower temperatures the proportion is much less.

According to Walker and Hambly, the rate at which ammonic cyanate undergoes conversion into urea is such that it cannot be supposed that only a single changing substance is present in the solution, the inversion proceeding at a "bimolecular" rate: in other words, the single molecule of cyanate is not changed directly into the single molecule of urea. According to Fawsitt, the hydrolysis of urea follows the unimolecular law.

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[*] Comp. Fawsitt, 'Zeit. Phys. Chem.,' 1902, vol. 41, p. 601.

[†] 'Chem. Soc. Trans.,' 1895, p. 746.

The observed rate of change may be accounted for in two ways: either on the assumption that when the cyanate is dissolved in water it is in part resolved into cyanic acid and ammonia; or on the assumption that the cyanate is dissociated electrolytically into the two ions, the ammonic ion and the cyanic ion—the process measured in either case being the rate at which the reverse interaction takes place by which the cyanate is reconstituted and transformed into urea.

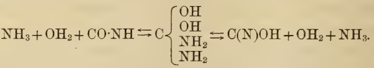
Walker and Hambly have proved that whereas a neutral salt such as potassic sulphate and also ammonia have but a slight retarding effect, ammonic sulphate and potassic cyanate both hasten the rate at which cyanate is changed into urea.

The same observers have shown that a variety of non-electrolytes—methylic and ethylic alcohol, glycol, glycerol, acetone and cane sugar—have also a marked influence in accelerating the rate of change; they express themselves unable to account for the acceleration, though they have no doubt as to the interpretation to be given to the results they obtained with electrolytes, as they remark "On no other theory (than that of electrolytic dissociation), as it appears to us, can even a qualitative explanation of our results be given."

We venture to differ. To us it appears easy to interpret the results on ordinary simple principles without invoking the aid of a mysterious, electrolytic-ionic dissociation. It is sufficient to assume that ammonic cyanate undergoes hydrolytic dissociation (dissociation through the agency of water) into cyanic acid and ammonia and that urea is formed by the gradual interaction of these substances and water; the effects electrolytes produce in altering the rate of change may be accounted for as easily on this assumption as on that of ionic dissociation favoured by Walker and Hambly. The fact is the observations may be interpreted from either point of view: as in so many other cases, the ionic explanation is merely an ornate paraphrase of the ordinary chemical point of view.

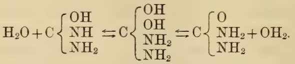
Walker and Hambly offer no explanation of the formation of urea. It appears from their observations that when ammonic cyanate is dissolved in water it is to some extent resolved into its components; the slowness with which urea is formed at moderate temperatures is attributable, it may be supposed, to the slowness with which cyanic acid, ammonia and water interact and with which the product of their interaction gives rise to urea, the slowness arising perhaps from the frequent reversals which attend the changes. In any case, the process is to be regarded as one of considerable complexity, in no way as a mere structural change in a single molecule.

The uncertainty we feel as to the structure of cyanic acid is of little consequence in such a discussion, as it is probable that a solution of the substance contains both metameric forms of the compound and that each of these can pass over into the other as required. Such molecules would be converted by the combined action of ammonia and water into a dihydroxydiamino-methane or diamino-methanediol:



$$NH_3+OH_2+CO{\cdot}NH \rightleftarrows C\left\{\begin{matrix}OH\\ OH\\ NH_2\\ NH_2\end{matrix}\right. \rightleftarrows C(N)OH+OH_2+NH_3.$$

The dehydration of the diol might well occur in either of two ways and give rise to one or other of the two forms of urea:



$$H_2O+C\left\{\begin{matrix}OH\\ NH\\ NH_2\end{matrix}\right. \rightleftarrows C\left\{\begin{matrix}OH\\ OH\\ NH_2\\ NH_2\end{matrix}\right. \rightleftarrows C\left\{\begin{matrix}O\\ NH_2\\ NH_2\end{matrix}\right. +OH_2.$$

Such dehydration changes would take place, we may suppose, the more readily the more concentrated the solution and the higher the temperature; they would be promoted by the presence of salts, as these would "concentrate" the solution; and would also be promoted by non-electrolytes, as these would raise the osmotic tension in the solution and render the water itself more active as a dehydrating agent.

Ammonic salts and cyanates, in common with all other salts, would act as "dehydrating" agents and therefore as accelerators. The effect produced by salts other than these, however, would be two-fold: whilst active as accelerators, they would also tend to confer stability on the cyanate, in virtue of the occurrence of changes such as the following:—

$$NH_4{\cdot}CNO+KCl \rightleftarrows KCNO+NH_4Cl.$$

Ammonia, being so very weak a base, would have a relatively slight effect in promoting the formation of the diamino-derivative and increasing the amount of change; on the other hand, by increasing to some extent the activity of water, it would to some extent perhaps also counteract its own tendency to increase the proportion of diamino-derivative produced, whilst it would in some degree promote dehydration changes. It may be expected, therefore, that the aggregate effect of ammonia would be slight.

Hydrolysis of Urea by Soja Urease.—It is well known that though urea does not undergo hydrolysis under ordinary conditions, it rapidly disappears from voided urine—the reason being that organisms which cause its destruction soon develop in the liquid. The hydrolysis is affected by *urease*, an enzyme present in *Micrococcus urae* and a number of other organisms. The literature of the subject is very fully brought together and discussed in Reynolds Green's 'Soluble Ferments and Fermentation' and similar works.

No simpler case of hydrolysis by an enzyme than that of urea is known. On this account we have long desired to include the interaction of urea and urease in our series of studies of enzymes. At various times, we have attempted to obtain the necessary enzyme for the work but the difficulties we encountered have been greater than we anticipated: little progress was made, therefore, until we were able to avail ourselves of the discovery made by Takeuchi, in 1909,* that the Soja bean (*Glycine hispida*) is particularly rich in urease.

Preparation of Enzyme.—To prepare a solution of the enzyme, the Soja beans are twice ground in a coffee mill and the meal extracted with petroleum spirit, the extraction being repeated six times; usually the petroleum is left in contact with the meal during about 45 minutes and then removed by filtration under reduced pressure. The operation is carried out in a large glass separating funnel. The washed meal is spread out during a night on glass plates to allow adherent petroleum to evaporate. Next morning it is mixed with five times its weight of water and a little toluene. After 24 hours, the liquid is separated from the meal by filtration through a large Buchner funnel; from 60 to 70 per cent. of liquid is recovered.

The solution thus prepared has a considerable degree of activity, 20 c.c. mixed with an equal volume of a solution containing about half a gramme of urea sufficing to hydrolyse the urea within half an hour at 20°.

The solution contains a large amount of albuminous matter. The endeavour has been made to purify the enzyme by precipitating with alcohol and extracting the precipitate with water; as the extract prepared in this manner is very weak in comparison with the new extract, we have preferred to use the latter.

The fresh extract is faintly acid; when kept, it increases in acidity and a coagulum is deposited; at the same time, its hydrolytic activity falls, the fall being considerable when the precipitation sets in. The changes that take place in the activity of the solution will be considered later on.

Method of Determining Activity of Enzyme.—We have found the simplest method of determining the activity of urease to be to digest the solution of urea with the enzyme and subsequently neutralise the ammonia that is formed by an excess of decinormal acid, then boil during two to three minutes to expel carbon dioxide, finally titrating with baryta solution. The errors incidental to this method do not appear to be greater than those associated with more elaborate methods.

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* 'J. Coll. Agric. Imp. Univ. Tokyo,' 1909, vol. 1, pp. 1—14.

In carrying out the experiments, as a rule, the mixtures studied were contained in Jena flasks provided with rubber stoppers. In some cases, when it was thought that ammonia might be lost by volatilisation, the stopper was provided with a short length of glass tubing to which a piece of rubber tubing carrying a screw clip was attached; the mixture having been introduced, the pressure within the flask was slightly reduced and the clip then screwed up; at the close of the experiment, by attaching the rubber tube to the burette and allowing acid to run in, it was possible to prevent any escape of ammonia. In some experiments in which a large number of samples were removed in rapid succession, the mixture was contained in a flask attached to an automatic pipette and the liquid forced up into this latter by air pressure—both flask and pipette being in the incubator and the pressure operated from outside.

Selective Activity of Urease.—Takeuchi has tested the action of Soja urease on a considerable number of nitrogen compounds, namely alanine, allantoin, arginine, benzamide, glycine, guanine, hippuric acid, histidine, kreatinine, leucine, tyrosine and uric acid; none of these was affected. He states that biuret is slightly attacked but his figures scarcely justify the conclusion.

We have thought it desirable to study the behaviour of substances more closely related to urea and have therefore subjected the various substituted ureas to the action of urease.

A convenient and striking way of demonstrating the hydrolysis of urea by Soja urease is to place a small quantity of a 5 per cent. solution of urea in a flat glass dish or on a white plate, then to add a drop or two of an alcoholic solution of phenolphthalein and dust in a quantity of the bean meal free from oil or even raw meal. In a very short time, especially if the liquid be slightly warmed, the colourless solution assumes a pink and then a full rose-red colour. If the experiment be made with either methylurea or ethylurea, no such effect is observed.

In a first series of experiments with various substituted ureas in which solutions of the ureas were digested with the solution of urease and then subjected to distillation with steam, the distillates obtained were so slightly alkaline that it was evident that little if any action had taken place. A second set of observations was then instituted with 2M/5 solutions, prepared at 25°, of methylurea, *s*-dimethylurea, *as*-dimethylurea, ethylurea and *s*-diethylurea and one of biuret prepared at 35° (as the biuret crystallised out at 25°).

From each of the solutions, 20 c.c. was measured into each of two 300 c.c. round Jena flasks, one containing 20 c.c. of Soja extract, the other 20 c.c. of water. The 12 flasks containing these mixtures and another containing

20 c.c. of Soja extract diluted with 20 c.c. of water were closed with indiarubber stoppers through which passed glass tubes provided with indiarubber tubes and screw clips. The flasks were exhausted slightly and then kept in incubators at 25°. After 24 hours, the contents of each flask was heated to boiling and distilled in a current of steam during 15 minutes, excessive frothing of the solutions containing enzyme being prevented by addition of a few drops of olive oil. Each distillate was collected in 10 c.c. of a standardised solution of chlorhydric acid; the distillate was then heated to boiling to expel carbon dioxide, cooled and titrated with baryta solution, using litmus as indicator. The results are given in Table I.

Table I.—Action of Soja Urease on Substituted Ureas.

Substituted urea.	Standard acid neutralised by distillate from		Amount of hydrolysis of		
			Urea alone.	Urea + enzyme.	
	Urea alone.	Urea + enzyme.		Uncorr.	Corrected for enzyme.
	c.c.	c.c.	per cent.	per cent.	per cent.
Methylurea	0·0	0·45	—	0·3	0·15
s-Dimethylurea ...	0·5	0·8	0·3	0·5	0·4
as-Dimethylurea ...	0·2	0·2	0·1	0·1	0·0
Ethylurea............	0·6	1·65	0·4	1·0	0·9
s-Diethylurea	0·25	0·25	0·15	0·15	0·03
Biuret	0·35	0·3	0·15	0·12	0·05

The evidence thus obtained appears to us to be conclusive proof that urease is capable of acting only on urea itself—in other words, it exercises a purely selective effect and must, like other enzymes, in some way correspond very closely in structure with the hydrolyte urea with which alone it is in correlation.

The value of such correlation will be realised more fully perhaps when it is stated that either acid or alkali of normal strength may be kept in contact with urea during several days at 25° without any perceptible degree of hydrolysis being effected, though in the presence of the enzyme the urea is resolved rapidly into ammonia and carbonic acid.

Time Rate of Change of Urea by Soja Urease—Effect of Concentration.—In our earliest experiments we were struck by the fact that the graphs representing the course of the change were of extreme flatness in comparison with those representative of the action of other enzymes. On attempting to effect the hydrolysis at a constant rate by means of a very small proportion of enzyme, using M/5 solutions of urea, we found that though, in the later periods, the amount of change taking place was approximately proportional to the time, the extent to which hydrolysis took place during the first hour

Table II.

Time.	Percentage of urea hydrolysed	
	By 2 c.c. of Soja extract per 100 c.c.	By 4·5 c.c. of Soja extract per 100 c.c.
15 mins.	2·6	—
30 „	3·8	12·6
45 „	4·9	16·3
1 hour	6·1	21·3
1·5 „	7·9	28·4
2·0 „	8·6	35·0
2·5 „	9·7	40·9
3·0 „	11·9	46·4
4·0 „	13·0	56·8
4·5 „	13·8	61·3
5·0 „	14·3	65·4
6·0 „	16·2	73·7
6·5 „	17·1	77·2
24·0 „	37·1	100·0
144·0 „	52·9	

Table III.—Hydrolysis of Urea in different Concentrations by the same amount of Urease.*

Time.	Volume of standard acid solution neutralised by 10 c.c. samples of solution.		
	M/5 urea solution.	M urea solution.	5M urea solution.
	c.c.	c.c.	c.c.
5 mins.	4·95	4·2	2·6
10 „	7·8	—	4·0
15 „	10·35	9·4	5·2
30 „	16·9	15·55	7·6
45 „	22·4	20·85	—
60 „	27·1	25·75	11·9
75 „	31·25	—	—
90 „	35·25	—	15·65
2 hours	39·14	—	19·1
3 „	—	61·8	25·4
5 „	—	85·35	36·4
7 „	—	108·4	46·2
25 „	—	196·85	—
49 „	—	—	93·8
Volume of acid neutralised by 10 c.c. on complete hydrolysis	39·5	197·5	987·5

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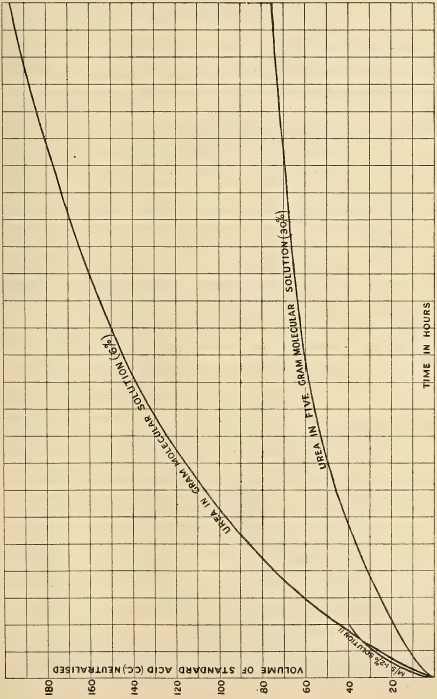
* In these experiments, instead of boiling the solution after adding acid, the carbon dioxide was expelled by means of a current of air, in order to avoid the error arising from the hydrolysis of urea in concentrated solutions by the excess of acid.

was several times that effected during subsequent similar periods. Experiments with larger proportions of enzyme gave results which showed less disproportion (Table II). In the case of more concentrated solutions, on



increasing the concentration of the urea, the amount of action taking place was diminished rather than increased, little advantage being obtained by using solutions stronger than M/5. This remarkable result is brought out very clearly in Table III and in the graph on p. 117.

It will be noted that up to the time of complete hydrolysis of the urea present in the M/5 solution practically the same amounts of change took place in this solution and in that which was five times as strong; moreover, that in the still stronger 5M solution less than half as much urea underwent conversion as was hydrolysed in that of one-fifth the concentration.

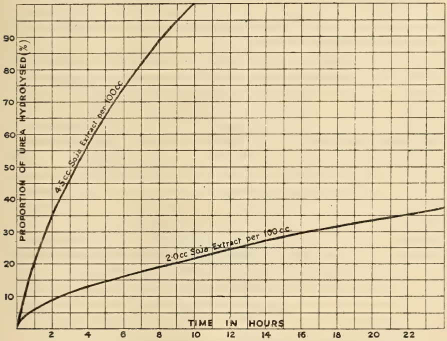
Influence of the Products of Change.—As it appeared probable from these observations that the products of change were exercising a marked influence, experiments were made to test the influence of ammonia. The effect produced by this substance was found to be very considerable: not only was the rate of hydrolysis limited but the graphs representing the course of the change appeared flattened out from the outset, not curved as they are when no ammonia is added.

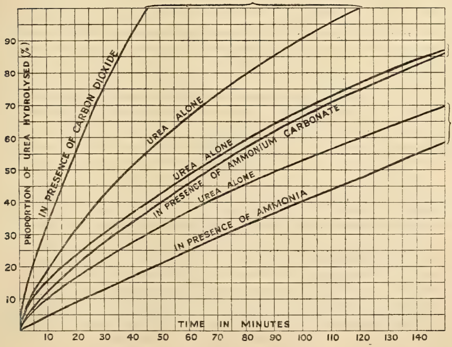
In an experiment in which 10 per cent. of the ammonia producible by the hydrolysis of the urea used was added at the outset, a small proportion of enzyme being used, the change followed an almost linear course during the first two hours, until over 50 per cent. was hydrolysed (Table IV, and the lowest pair of curves in the lower graph on p. 119).

Table IV.—Hydrolysis of Urea in the presence of Ammonia and Ammonium Carbonate.

Time.	Percentage of urea hydrolysed in solution containing			
	Urea alone.	Urea + ammonia.	Urea alone.	Urea + ammonium carbonate.
5 mins.	6·3	3·0	11·6	7·4
10 „	9·9	4·4	15·1	12·3
15 „	13·9	6·4	18·3	16·7
30 „	21·8	12·5	29·4	27·2
45 „	27·4	16·3	39·6	37·0
60 „	36·8	25·7	47·4	46·9
75 „	43·4	31·4	56·2	54·5
90 „	49·2	36·7	63·2	62·1
105 „	—	—	71·3	69·3
120 „	58·6	47·5	76·0	75·5
150 „	70·1	58·3	87·5	87·6
21 hours	97·3	97·6	95·5	95·5

When ammonic carbonate (equivalent to one-tenth of the urea used) was substituted for ammonia, though at first a decided retardation was obvious this was not so decided as in the case of ammonia and gradually (as ammonia was formed) the two solutions approximated in behaviour—so that the change as a whole did not proceed at a linear rate. These results are indicated by the middle pair of curves in the lower graph on this page.





Again, when at the end of an experiment (when the action was complete and therefore the products of change were present in a proportion equivalent to the urea originally present) a fresh quantity of urea, equal to that originally taken, was added, the rate at which hydrolysis took place initially was about one-third of that first observed and the time required to effect complete hydrolysis was more than doubled.

In another experiment, in which a third portion of urea was introduced, hydrolysis took place at a very slow rate, 1·2 per cent. being changed after 30 minutes in this third stage instead of 10 per cent. in the second stage and 25 instead of 97 per cent. at the end of six hours.

Inasmuch as ammonia had so much more influence in retarding change than the carbonate, it appeared probable that the effect was attributable to its acting as an alkali. But it was conceivable that by favouring the activity of proteoclastic enzymes associated with the urease it might promote the destruction of the enzyme rather than retard its action.

Stability of the Enzyme.—To test the stability of the enzyme under various conditions, 25 c.c. of an extract prepared in the manner previously described was diluted to 250 c.c. with water; a second 25 c.c. was diluted to 250 c.c., sufficient ammonia being added to make the solution N/500 NH_3 in concentration; a third 25 c.c. was similarly diluted with water and acidulated to be of the strength N/500 HCl; a portion of the extract was set aside undiluted. The activity of each of these solutions towards urea was tested as soon as they were made and after various intervals and in the case of the acid and alkaline liquids, two experiments were made with each solution—

Table V.

Enzyme solution.	Percentage of urea hydrolysed in mixtures of 20 c.c. enzyme solution and 20 c.c. 2M/5 urea solution in 1 hour at 25°.							
	Initial.	1 day.	3 days.	4 days.	7 days.	11 days.	17 days.	60 days.
Aqueous solution of 10 per cent. of Soja extract [kept]	25·3	25·3	24·3	—	23·8	23·3	21·5	9·6
Ditto [made at time of experiment]	25·3	25·7	24·9	—	23·7	23·1	21·5	10·4
N/500 NH_3 solution of 10 per cent. of Soja extract [kept]	25·9	25·2	24·1	—	23·3	22·7	—	—
Ditto [neutralised at time of experiment]	25·9	25·3	24·6	—	22·7	23·7	22·8	11·0
N/500 HCl solution of 10 per cent. of Soja extract [kept]	25·9	25·0	21·4	19·6	17·3	15·5	—	—
Ditto [neutralised at time of experiment]	25·5	24·1	21·0	18·9	17·1	14·5	11·9	3·1

one with the unneutralised liquid, the other after neutralising it by adding the equivalent amount of alkali or acid. The results are given in Table V. It will be noticed that the acid but not the ammonia had a distinct influence in promoting decay of the enzyme.

The effect was found to be more marked in more strongly acid or alkaline solutions. The results given in Table VI show, however, that though the presence of ammonia influences the activity of the enzyme, decay takes place only gradually.

Table VI.

Enzyme solution.	Percentage of urea hydrolysed in mixtures of 20 c.c. enzyme solution and 20 c.c. 2M/5 urea solution in 1 hour at 25°.			
	Initial.	1 day.	2 days.	4 days.
Aqueous solution of 10 per cent. of Soja extract [kept]	26·4	24·9	25·3	21·4
N/25 HCl solution of 10 per cent. of Soja extract ……	2·6	—	—	—
N/25 NH_3 solution of 10 per cent. of Soja extract [kept]	19·2	15·2	13·1	8·9
Ditto neutralised at time of experiment ……………	—	—	—	8·8
Ditto saturated with CO_2 at time of experiment……	—	—	—	18·3

The experiments appear to justify the conclusion that the effects produced initially by ammonia are at least mainly due to the influence it exerts on the change itself and that there is no reason to suppose that the enzyme is destroyed by it, to any serious extent, either in the first instance or during the course of an experiment lasting a short time.

Influence of Carbon Dioxide.—Inasmuch as carbonate of ammonia has a very slight inhibiting effect in comparison with that of ammonia, it was to be supposed that carbonic acid would serve to neutralise, almost if not entirely, the effect ammonia produces. Having formed the opinion that the ammonia acted as an alkali and was in some way opposed to the urea, we therefore made the experiment in the full expectation that it would exercise a positive influence; we were not prepared, however, to find that carbonic acid has so marked an influence in promoting and increasing the activity of the enzyme as our experiments show that it has. The results of a pair of experiments are represented in the uppermost pair of curves in the lower graph on p. 119 and in Table VII.

In making these experiments, the behaviour of an M/5 solution of urea containing 25 c.c. of Soja extract was contrasted with that of a solution of the same strength prepared by saturating the liquid with carbon dioxide prior to adding the enzyme and then maintaining the passage of a current

Table VII.—Hydrolysis of Urea in the presence of Carbon Dioxide.

Time.	1st experiment. Percentage of urea hydrolysed in		2nd experiment. Percentage of urea hydrolysed in	
	Aqueous solution.	Carbonic acid solution.	Aqueous solution.	Carbonic acid solution.
mins.				
5	12·3	18·2	11·7	19·9
10	19·5	32·3	19·0	32·5
15	26·6	—	25·5	44·0
20	—	54·3	—	54·0
30	—	73·4	41·8	74·0
40	48·9	—	—	91·9
45	—	95·5	54·0	—
60	65·3	95·7	65·5	99·0
75	76·0	96·5	75·9	—
90	84·5	98·0	85·4	99·6
105	—	98·5	—	—
120	98·5	—	98·5	—

of the gas through the solution while it was heated in the incubator in proximity to the unheated solution.*

A similar effect is produced by the "amino-acid" glycine—thus the activity of a solution of the enzyme which produced 24 per cent. of change was raised to 35·3 per cent. by adding sufficient glycine to make it a 2N/5 solution of this substance.

Influence of Salts and Non-electrolytes.—As ammonia salts hasten whilst salts of the alkali metals retard the formation of urea from cyanate of ammonia, it was important to contrast the effect produced by ammonium

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* *Note added February* 23.—The following series of results obtained recently are of interest as confirming those quoted in the communication, especially as the experiments were carried out simultaneously and with the same sample of enzyme. The very rapid rate of change in the presence of carbonic acid towards the close of the period of hydrolysis is noteworthy. A very striking diagram is obtained by plotting graphs from the figures given.

Percentage of Urea Hydrolysed in M/5 Solutions when alone or in presence of 1/10th equivalent of ammonic carbonate or 1/10th equivalent of ammonia, and in a solution saturated with carbon dioxide.

Minutes	5	10	15	20	25	30	40	45	50	60	75	90	120	150
Urea+ammonia	4·0	9·0	13·3	—	—	25·4	—	36·7	—	47·6	—	68·0	83·9	97·8
Urea+ammonic carbonate	8·6	16·4	22·2	—	—	37·6	—	48·8	—	59·4	—	79·8	96·8	—
Urea alone	11·8	19·2	25·5	—	—	41·5	—	54·4	—	65·1	74·4	83·2	97·0	—
Urea+carbon dioxide	20·9	30·0	42·3	54·2	65·0	75·9	98·5	—	99·3	—	—	—	—	—

and other chlorides on the hydrolysis of urea by urease. Potassium and sodium chloride act alike, both retarding the rate of change to a moderate extent. Ammonium chloride has a very slight accelerating effect, about equal to that exercised by glucose; methylurea, on the other hand, has as great an effect in retarding change as have potassium and sodium chloride. The various substances were present in the same molecular proportion as the urea at the outset. The results are represented in the set of graphs on p. 125 and in Table VIII.

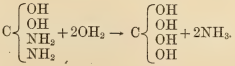
Nature of the Process of Hydrolysis.—In view of the results we have obtained, there is reason to suppose that urease, like all other enzymes hitherto examined, is a selective enzyme—that is to say, an enzyme which fits the hydrolyte of which it is the specific hydrolyst. This hydrolyte may well be the hydrated form of urea, $C(OH)_2(NH_2)_2$, rather than urea itself.

The question to be considered is the nature of the change by which the urea is broken down. It seems to us that there is no reason to suppose that it is resolved otherwise than into carbonic acid and ammonia by a direct process of hydrolysis; the only intermediate product the formation of which should be considered is carbamic acid.

It has been argued by Fawsitt (*loc. cit.*) that the formation of carbonate from urea involves the intermediate conversion of the urea into cyanate but it may be contended that his argument is inconclusive and indeed irrational. In the light of modern knowledge, neither the conversion of urea into ammonic cyanate nor the reverse change can be looked upon as a simple process of internal molecular rearrangement; in both cases doubtless, a series of changes take place.

The formation of urea from the cyanate is probably a process involving both hydration and dehydration and may be represented, with some degree of probability, in the manner pictured in the equations on p. 112.

The formation of carbonic acid and ammonia from urea, on the other hand, is clearly a process of hydration and hydrolysis. It is conceivable and in our opinion probable that both changes—that into cyanate and that into carbonate—have their origin in the same substance, the hydrated compound which may be supposed to be present in a solution of urea $C(OH)_2(NH_2)_2$. Whilst this compound can give rise to cyanic acid, if deprived of ammonia and hydrone (OH_2); if hydrolysed, it can give rise to orthocarbonic acid and ammonia—

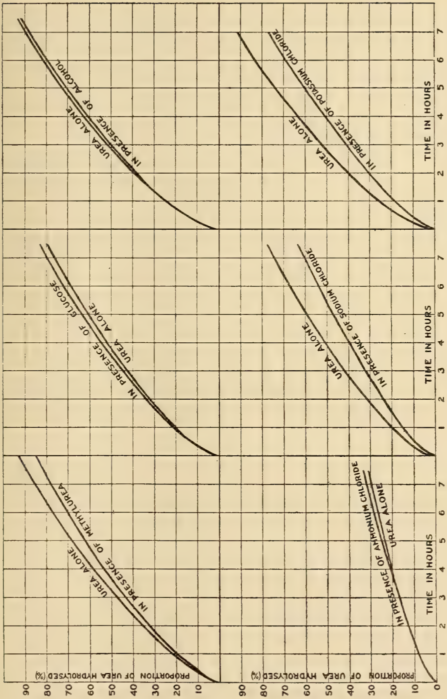


$$\mathrm{C}\begin{cases}\mathrm{OH}\\ \mathrm{OH}\\ \mathrm{NH_2}\\ \mathrm{NH_2}\end{cases}+2\mathrm{OH_2}\rightarrow \mathrm{C}\begin{cases}\mathrm{OH}\\ \mathrm{OH}\\ \mathrm{OH}\\ \mathrm{OH}\end{cases}+2\mathrm{NH_3}.$$

Table VIII.—Hydrolysis of Urea in the presence of Salts and Non-Electrolytes.

Time.	Percentage of urea hydrolysed											
	Alone.	In presence of methylurea.	Alone.	In presence of glucose.	Alone.	In presence of alcohol.	Alone.	In presence of NH_4Cl.	Alone.	In presence of NaCl.	Alone.	In presence of KCl.
hours.												
0·5	12·4	12·2	12·4	12·5	14·6	14·5	5·1	4·9	12·5	9·8	14·8	11·4
1·0	20·7	20·2	20·2	20·3	23·5	23·6	7·7	7·9	19·3	14·5	24·8	18·5
1·5	28·5	26·9	26·6	27·1	31·6	31·7	10·3	10·1	25·8	20·2	32·4	24·7
2·0	35·1	32·6	32·5	33·0	38·6	38·4	12·7	12·7	31·3	24·3	39·4	30·2
2·5	41·4	38·4	38·0	38·7	45·3	44·2	14·7	15·1	36·7	28·5	45·9	36·2
3·0	—	—	43·0	44·2	51·1	50·6	—	—	41·8	32·8	—	—
4·0	58·3	53·8	—	—	—	—	20·5	20·5	—	—	63·4	51·1
4·5	63·7*	58·2	56·9	58·5	67·0	66·1	22·2	22·4	54·5	43·4	—	—
5·0	67·8	62·3	61·0	63·1	72·1	71·3	23·9	24·4	58·8	47·2	73·6	60·6
5·5	72·4	66·4	65·2	67·0	76·0	75·5	25·2	26·5	62·8	50·6	—	—
6·0	77·0	70·8	69·3	73·5	80·6	80·1	26·0	27·4	66·5	53·6	82·4	69·1
6·5	80·9	74·5	72·9	75·2	84·8	84·7	28·1	29·7	70·1	57·0	—	—
7·0	84·9	78·5	76·6	78·8	89·2	88·1	—	—	73·9	60·1	90·7	76·5
7·5	—	—	80·1	81·9	93·0	92·2	31·2	33·0	77·2	63·4	—	—
8·0	92·3	84·7	—	—	—	—	—	—	—	—	—	—
24·0	99·8	100·7	—	—	—	—	70·4	73·1	—	—	99·2	99·4
24·5	—	—	99·9	99·9	99·0	99·2	—	—	99·6	99·4	—	—

* Sample taken after 4 hrs. 35 mins.



Hydrolysis of Urea in presence of Salts and Non-Electrolytes.

The function of urease would seem to be to determine the change in this latter direction: in other words, to condition the direct hydrolysis of urea and thereby prevent its reversion into cyanate.

It is necessary to account, on this assumption, for the effects produced by salts and non-electrolytes. The marked inhibiting effect of ammonia and the slight effect produced by ammonium chloride in hastening hydrolysis are hardly to be accounted for on the assumption that these compounds directly affect the stability of the urea: if we are not mistaken, according to current views, it is rather to be supposed that the ammonium salt would exercise the greater effect in repressing hydrolysis and the alkali ammonia should tend to promote hydrolysis.

We are inclined to think that an explanation is to be found by regarding the enzyme as a feebly acidic substance and that, in order that it may condition the change, it must unite with the feebly basic substance urea—the effect of a more basic substance such as ammonia would be to interfere with such union and consequently to retard change. Carbonic acid, by fixing the ammonia, would tend to promote the action of the enzyme from this point of view by leaving it free to act as hydrolyst. To account for the extraordinary retardation effected by urea itself, it must be supposed that the alterations in the osmotic conditions which are the consequence of an increase in the concentration of the solution in some way affect the rate at which change can take place; it may be that the proportion of urea effectively hydrated diminishes as the concentration is increased—that is to say that the compound $C(OH)_2(NH_2)_2$ is formed in diminishing proportion as the concentration is increased. The influence of non-electrolytes is at least in part to be accounted for on this assumption.

Urea is to be regarded as a compound which has very little tendency under ordinary conditions to become carbonate. Apparently it is protected from decomposition by its tendency to become cyanate; on the other hand, the cyanic acid which is formed from it when the change occurs is protected by the presence of an equivalent proportion of ammonia and is protected also by its tendency to revert to urea. In other words, both urea and cyanate are protected from further change by the tendency each has to become the other. The production of a relatively large amount of carbonate when ammonic cyanate is taken instead of urea is perhaps due to the fact that the solution then contains a relatively much larger amount of free cyanic acid than is present in a solution of urea, cyanic acid (in absence of ammonia) being an unstable substance which is readily hydrolysed to carbonic acid. That is to say, carbonic acid may be formed in two ways, from cyanic acid and from urea: under normal conditions, in a solution of urea neither the cyanic acid nor the urea-hydrate which are contained in the solution undergoes decomposition into carbonic acid to an extent which is appreciable, being present only in minute amounts. Urease, by determining the resolution of the urea in an abnormal direction therefore serves a most important purpose.

In studying the phenomena, the mistake has been made hitherto of treating the changes attending the formation and destruction of urea from a point of view which has involved under-rating their complexity.

We venture to think that the peculiarities to which we call attention are not only noteworthy but should serve as a warning against the danger attending the attempt to formulate conclusions as to the nature of chemical changes without giving due weight to chemical considerations—a practice too frequently followed in these days.

It is proposed to extend the inquiry.

[The cost of this investigation has been in part met by a grant for which I am indebted to the Government Grant Fund of the Royal Society.—H. E. A.]

Certain Results of Drying Non-sporing Bacteria in a Charcoal Liquid Air Vacuum.

By S. G. Shattock, F.R.C.S., and L. S. Dudgeon, F.R.C.P.

(Communicated by Sir James Dewar, F.R.S. Received February 20,—Read March 14, 1912.)

The following experiments were undertaken with the object of ascertaining whether non-sporing bacteria, dried *in vacuo* and kept *in vacuo*, would survive those dried in and kept in the air, or, on the contrary, whether they would die more rapidly. The action of sunlight and of heat was tested, moreover, upon bacteria dried *in vacuo* and kept *in vacuo*, with a view of discovering how far such agencies might be lethal upon dried bacteria, if the latter were supposed present in a free state in interplanetary space.

In carrying out the work, we have had the invaluable advantage of Sir James Dewar's help, for of the several methods of drying *in vacuo*, by far the most efficient is that devised by him. This method is so well known to physicists that it will be enough to state here, that after the air of the vessel is exhausted by means of an air pump, the glass connection with the pump is sealed off in the blowpipe flame, and the exhausted chamber is deprived of its remaining gases through a second outlet communicating with a bulb containing cocoanut charcoal (previously freed from gases), which is submerged and kept in a Dewar vacuum flask of liquid air. The use of mercury was avoided in producing the initial vacuum, in order to exclude the presence of mercury vapour, which might in various ways invalidate the results of the experiments.

The apparatus was stored in a dark room to eliminate the action of light, and for the same purpose metal foil was wrapped around the tubes during the process of exhaustion with the pump. The charcoal at the temperature of liquid air has a remarkable capacity for gas absorption. The action of this substance was maintained sometimes for three days, sometimes for five, during which time the vapour of water distilled continuously, and was condensed on the walls of the condenser at −195° C. whilst the gases were absorbed by the charcoal. The way connecting the tube with the bulb of charcoal was then sealed off in the blowpipe flame, and the tubes were stored in light-tight photographic bags in a metal box, which was, furthermore, kept in a closed cupboard.

The possible ways in which the vacuum may suffer are (1) in sealing off the side outlet, by the evolution of gas from the heated glass; (2) owing to the presence of microscopic fissures at the junctions of the apparatus, especially if many chambers are connected up with the same charcoal receptacle; (3) by reason of the fact that glass is hygroscopic, and that even after a prolonged period of high exhaustion, water might fail to be removed, at the ordinary temperature, from the inner surface of the vacuum tube or from the glass slip inoculated with the organic film of peptone containing the micro-organism.

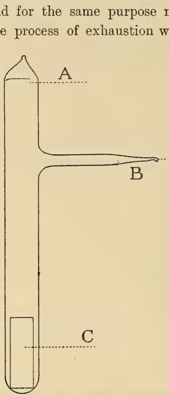


Fig. 1.—A. Open end of test-tube, sealed off after the insertion of the inoculated slip. B. Side tube, connected with exhaust pump, and also with glass bulb containing charcoal, etc. This is eventually sealed off in the blowpipe flame. C. Slip of thin glass with dried bacterial film.

The vacuum tubes employed were stout test-tubes of soft glass, furnished with a side channel by means of which they were connected (1) with the exhaust pump, and (2) with the bulb of charcoal. (See figure.)

The inoculations were made upon rectangular slips of thin glass (No. 2 microscopic cover) by means of a large platinum loop, the slips having been previously heated for one hour at 150° C., in the Petri dish in which they were afterwards inoculated. The immediate drying of the slips after the inoculation was carried out in the incubator at 37° C., in which they were kept for about 15 minutes.

Some of the slips were, within the course of an hour or so, transferred to the tubes, from which the air was thereupon exhausted; the rest were stored in the Petri dish in the dark. The bacterial suspensions first used were made by adding boiled water to a recent agar or jelly slope culture; all the later suspensions were cultures in peptone water (with 1-per-cent. sodium chloride).

In order to test the vitality of the bacilli after drying, whether *in vacuo* or in the air in the Petri dish, the slips were transferred to test-tubes of litmus glucose broth, and incubated for many days at 37° C. The broth tubes were proved to be sterile before use, by a preliminary incubation.

We may now proceed to detail the observations, and afterwards to comment on the results.

Bacillus coli.

The first experiments were made upon this bacillus, on October 14, 1910.

One vacuum tube was sealed off after three full days' connection with the charcoal tube, *i.e.* on the fourth day; and a second on the 6th day, the vitality of the micro-organism being tested as above described. In both cases it was dead. The control air-dried film was also found to be dead on the 4th day.

Bacillus typhosus.

Vacuum tubes were sealed off on the 4th day and on the 6th.

The bacillus in both proved to be dead. The control air-dried films were likewise dead on the 4th day. The exact day on which the air-dried micro-organism dies can be, of course, readily determined by transferring an inoculated slip daily to the broth medium.

Different strains or samples of the same bacillus vary within certain limits. In an observation so made, using for the inoculation a suspension of *B. typhosus* in water from an agar slant of 24 hours' growth, the bacillus died on the 4th day. In four experiments made with a 24 hours' growth in peptone water of *B. typhosus*, the micro-organism died, in one case, on the first day (*i.e.* within 24 hours); in two cases, on the 4th day; and in the fourth case on the 5th day.

Staphylococcus pyogenes aureus.

This is more resistent to desiccation, both *in vacuo* and in the air, than either of the preceding. In one experiment vacuum tubes were sealed off on the 4th day, and on the 6th. The staphylococcus grew abundantly in both cases within 24 hours. The control air-dried slips prepared at the same time grew equally rapidly after the same periods; further controls proved to be alive on the 16th and 23rd days, but when again tested on the 40th day the microbe was dead.

By means of a second series of observations the survival of the staphylococcus *in vacuo* on the 4th and 6th days was confirmed, the control slips in this case being likewise alive on the same days.

As it was thus clear that the staphylococcus would survive drying five days *in vacuo*, and also in the air, we proceeded to test its vitality for longer periods. Vacuum tubes were sealed off after five days' treatment with the charcoal and liquid air, and stored in the dark. The micro-organism from the tubes proved to be alive on the 33rd day, and so was the control. Further control slips were tested at different periods, and proved to be alive at nine weeks, four days; death had occurred at twelve weeks, four days, the microbe having died at some date in the interval.

As the control was now dead, a vacuum tube was tested 21 days later; the film proved to be sterile. In this experiment the air-dried slips died at some date between 9 and 12 weeks; those from the vacuum tubes died between 4 and 15 weeks.

(Two further vacuum tubes were tested at periods of 10 and 14 months; the slips from both were sterile.)

Bacillus pyocyaneus.

The results obtained with this bacillus are particularly interesting.

Films of the micro-organism, prepared as usual from a 24 hours' peptone water culture, proved to be alive on the 4th and 6th day when removed from vacuum tubes treated for these periods with charcoal and liquid air. Control air-dried slips were likewise alive at the same dates. The vitality was next tested for longer periods.

December 9, 1910.—A series of glass slips were inoculated. One slip was transferred to each of three tubes, which were thereupon exhausted, and sealed off after five days' treatment with charcoal and liquid air.

A control slip proved to be alive on the 6th day. One vacuum tube was opened on the 46th day—the bacillus proved to be alive; a control film was at this date dead.

A second vacuum tube was opened on the 116th day; the bacillus was still alive. The third of the tubes was opened on June 30 (seven months and seven days); within 48 hours an abundant growth of the bacillus (as confirmed by sub-culture) had occurred.

In confirmation of this remarkable longevity *in vacuo*, in a second set of observations, *B. pyocyaneus* was found to have remained alive from May 26, 1911, to December 16, 1911; a period of exactly the same length as the above.

One slip was tested on June 1, 1911, and was found alive. The growth obtained from the slip on December 16 was in every way characteristic. Within 48 hours, the litmus glucose broth was uniformly turbid, and was decolorised except for a zone at the free surface, which retained a violet tint; the top of the fluid was covered with a thin, unwrinkled, faintly greenish looking scum or zoogl&oelig;a. Sub-cultures carried to peptone water on December 18 showed within 24 hours general turbidity, and a delicate but well marked green coloration; a sub-culture from this carried to an agar slant gave within 24 hours a full growth, which within 48 hours had produced a typical green pigmentation of the medium in the neighbourhood of the culture.

The longevity of this bacillus *in vacuo* is the more remarkable since the micro-organism somewhat rapidly dies in air-dried films. Under the latter circumstances the date of death (as tested by daily transferring a slip to a broth tube) varies within certain limits; but we have never found the microbe alive after the 9th day.

Remarks.

Taking the results obtained by complete drying *in vacuo*, two obvious conclusions will appear. In the first place it is clear that the vulnerability of different bacteria varies within wide limits. And in the second, as the vitality of *B. pyocyaneus in vacuo* is notably prolonged beyond that of air-dried slips, the prolongation can only be due to the absence of chemical changes which obtain in the air, but are absent in the vacuum.

In regard to the first of these two results we may provisionally hold that it implies a difference in the composition, or the molecular construction of the protoplasm. It has a parallel in the difference of resistance to heat. The thermophilic bacteria thrive at a temperature of 70° C., whilst the common death point of other (non-sporing) forms, when suspended in fluid, ranges close about 60° C.

The different resistance of different bacteria to drying *in vacuo*, and to heat, indicates that the chemical constitution, or the molecular construction, of the protoplasm varies; that protoplasm is not a definite chemical substance, but one of varying range.

The selection of particular body-cells by particular poisons is one proof of such a difference in the same organism. It is probably correct, indeed, to hold that every functional difference amongst cells implies a protoplasmic one. *Quot actiones tot protoplasmata.* And the same of bacteria, physiologically considered. How long *B. pyocyaneus* will live in the dried state *in vacuo* is an interesting question, and we have a series of vacuum tubes sealed off to keep (excluded from the light) for protracted periods, with a view of testing it.

In the air-dried state *B. pyocyaneus* presents no particular longevity. Its death under these circumstances cannot be ascribed simply to its drying. The film may absorb moisture at intervals according to the saturation of the atmosphere, and the bacillus may be killed by a recurrent process of oxidation. Or dissociation may occur in some of the less stable constituents of the protoplasm. In the vacuum tube oxidation would be excluded, but dissociation or autolysis might occur, and eventually prove fatal.

In the case of *Staphylococcus pyogenes aureus*, which survives some weeks *in vacuo* and then dies, something of the latter kind may be assumed, by way of exclusion, to take place.

It may be pointed out that these differences in resistance to drying *in vacuo* cannot be ascribed to the chemical differences indicated by Gram's staining reaction. *Staphylococcus pyogenes aureus* (Gram positive) is, it is true, more resistent than *B. coli* or *B. typhosus* (both Gram negative) but the most resistent of all, *B. pyocyaneus*, is, like the least, Gram negative.

The ordinary microscopic examination does not reveal any recognisable change in bacilli which have been long dried. A dried peptone-water film of *B. pyocyaneus*, made November 26, 1910, and stored in the dark, in a Petri dish, showed, when stained with carbol fuchsin, and examined with 1/12 immersion, on June 15, 1911, nothing in which it would differ from a recently made preparation. And the same is true of *Staphylococcus pyogenes aureus*.

A similar film of *B. pyocyaneus* made in July, 1911, was examined in January, 1912, in a hanging drop, which was prepared by wetting the dry film with germ-free distilled water, and transferring a loop of the suspension to a cover-glass. The bacilli after imbibition of water were of full size, and perfectly normal in form.

The Persistence of Vitality in Bacteria in the Dried State in vacuo, *and the Question of their Resistance in this Condition to Physical Agencies.*

The most fascinating problem in connection with the vitality of bacteria *in vacuo* is the possibility of their interplanetary life.

That certain bacteria can survive in a fully dried state *in vacuo* is as important a fact in this connection as that so many may be frozen at the temperature of liquid air (−190° C.) without being killed.

Particulate life, whether microscopic or ultra-microscopic, if free under interstellar conditions, would exist *in vacuo*, and, either in a dried state, or impregnated with water, it would be cooled at a temperature certainly as low as that of liquid air.

The external agencies adverse to life in such circumstances resolve themselves into the action of the solar rays : heat, light, ultra-violet, and the corpuscular radiations.

Heat.

If the inoculated slips of *B. pyocyaneus* in sealed vacuum tubes be submitted to the action of heat, the vulnerability of the bacillus to this agency is not found to be lessened. It has long since been established that many bacteria, even of the non-sporing kinds, withstand a higher temperature in the dry state than in the wet. Suspended in a fluid medium, *B. pyocyaneus* is killed by a temperature of 60° C. after an hour's exposure.

In testing the effect of heat upon this bacillus in the dried state, we commenced with a temperature which is lethal to all non-sporing pathogenic bacteria, in order to ascertain whether its resistance *in vacuo*, if exalted, was exalted in a pronounced degree.

July 20, 1911.—A vacuum tube containing an inoculated slip of *B. pyocyaneus* which had been sealed off on July 17 and kept in the dark was baked in the hot-air oven for three hours, between 102° and 104° C.

No growth occurred from the slip when transferred to litmus glucose broth and incubated at 37° C.

A control slip, prepared on July 20, and baked simultaneously for the same time in a test-tube, over which a second larger tube was inverted, for protection, likewise proved to be sterile.

In the following experiment, the air-dried films of *B. pyocyaneus* were subjected to a temperature of 100° C. in a water bath, for considerably shorter periods.

January 6, 1912.—A series of slips were prepared from a 24 hours old peptone water culture of *B. pyocyaneus* in the usual manner. Three were then transferred, each to a long sterilised test-tube of thick glass ; the tubes were thereupon sealed in the blowpipe flame by heating each at a considerable distance below the open end. The three tubes were, in the next place, submerged by means of strips of lead in a vessel of warm water and boiled for 15, 30, and 60 minutes. One end of the tube was cut off with a file, and the slip transferred to a tube of litmus glucose broth, and incubated at 37° C.

After this, a control slip from the Petri dish was placed into another tube of the same medium and incubated with the rest. No growth ensued in the case of any of the three heated slips; the control gave a full growth of *B. pyocyaneus* within 48 hours.

January 13, 1912.—It being thus clear that the air-dried film of *B. pyocyaneus* is killed by 15 minutes' heating at 100° C., a vacuum tube containing a slip of this bacillus, which had been sealed off August 1, 1911, after the usual treatment, was boiled for 45 minutes.

The time selected was triple that of the minimum which had been found lethal in the case of the air-dried film. The purpose of this was to ensure that the film was raised to the temperature of 100° C., seeing that its heating *in vacuo* would be delayed, owing to the absence of gas convection, and could occur only by radiation, and conduction from the heated tube to the edges of the glass slip in contact with its inner surface. After being boiled for 45 minutes, the tube was opened with the usual precautions, and the slip transferred to litmus glucose broth, and incubated at 37° C.

A second vacuum tube was then opened as a control, and the contained slip transferred to another tube of the same medium; the vacuum tube was one of four which had been sealed off at the same time, viz., August 1, 1911; the slips in these four tubes had been inoculated from the same peptone water culture of *B. pyocyaneus.* The slip from the boiled tube proved to be sterile; that from the control tube gave an abundant growth within 48 hours, which was identified by sub-culture as *B. pyocyaneus.*

Proceeding with temperatures lower than 100° C., air-dried slips of *B. pyocyaneus* (prepared as in all other cases, for the immediate occasion) were treated in sealed tubes by submersion in water at 80° C., after having been warmed for 15 minutes at the innocuous temperature of 40° C. One slip was heated for 30 minutes, one for 60. Both proved to be sterile. The air-dried, unheated, control slip, which was transferred to broth after the completion of the heating of the others, gave a full growth.

By the same method it was found that temperatures of 76° C. and of 65° C. are lethal when maintained for an hour.

The results show that in the case of *B. pyocyaneus* the lethal temperature is practically the same, whether the micro-organism is subjected to it in the wet state or the dry.

We terminated these observations by heating a vacuum tube containing a slip of *B. pyocyaneus,* sealed off August 1, 1911, for three hours at 65° C. in a water bath. The film proved to be dead. The control, unheated, film in another vacuum tube of the same batch (August 1, 1911), tested on the same day, gave a typical and abundant growth within 48 hours.

It appears plainly, therefore, from these observations that the resistance of

B. pyocyaneus to the action of heat is not heightened when the micro-organism is exposed to this agency in the dried state *in vacuo*.

Sunlight and Ultra-violet Rays.

The lethal agent of first importance, however, in connection with a supposititious interplanetary life in a free or unincluded condition, is sunlight. As this reaches the deeper strata of the atmosphere, the ultra-violet rays are filtered out of it. Notwithstanding this, its lethal properties in regard to bacteria have been long established; even endospores are killed by it.

Is the dried *B. pyocyaneus* killed, *in vacuo*, by the action of sunlight? This question must be answered in the affirmative.

In the following observations the action of the sun's heat was eliminated by submerging the tubes in a large shallow glass dish of water, by means of strips of lead wrapped round their ends. The glass dish was raised some way from the ground by allowing its two ends to rest upon the edges of two stools. The apparatus was exposed on a roof during the unusually bright weather of July and August, 1911. The inoculated glass slip was displaced by tilting the tube, to the centre of the latter, and in such a way that the flat surface lay upwards.

July 22, 1911.—A vacuum tube containing a slip of *B. pyocyaneus*, which had been sealed off on July 17, 1911, after three days' treatment of the vacuum by means of charcoal and liquid air, was exposed, as described, from 11.15 A.M. for six hours, the sunlight being intense and not at any time interrupted. The tube was then opened, and the slip transferred to a tube of litmus dextrose broth. No growth ensued.

July 20.—A second vacuum tube, sealed off on July 17, was exposed, as detailed, from 1 P.M. for four hours. For portions of the time the sun was behind clouds.

July 21.—The tube was a second time exposed (after being stored in the dark) from 11 A.M. for six hours, the sunlight being on this occasion intense and uninterrupted. The slip was then removed and transferred to litmus glucose broth. No growth ensued.

A control tube made by inserting an air-dried slip prepared from a peptone water culture on the morning of the experiment, July 20, into a test-tube of stout glass and sealing the end in the blowpipe flame, was exposed for the same two occasions in the same dish of water: the slip, on being transferred to a tube of the same medium, viz., litmus glucose broth, proved sterile.

Another control tube was exposed on July 20 for four hours (sunshine interrupted), and the slip of *B. pyocyaneus* then transferred to a tube of litmus glucose broth; it gave a characteristic growth.

As a comparable test of the action of sunlight upon *B. pyocyaneus* in the moist state, an agar slant from which the water of expression had gone, was inoculated, without disturbing the surface, with a 24 hours' growth of the bacillus in peptone water. The end of the tube was sealed in the blowpipe flame, and the tube was exposed on July 20, in the same dish of water as used on that day for certain of the observations already recorded, for four hours' sunshine (interrupted). The end of the tube was then removed, and the agar medium covered by pouring in litmus glucose broth; the tube was thereupon plugged with sterilised wool.

A growth of the bacillus (identified by sub-culture) followed in this case as in that of the dried film exposed for the same time on the same day.

A second control made in precisely the same way upon an agar slant, from a 24 hours' growth in peptone water, and exposed on July 21 for five and a-half hours' uninterrupted sunshine (from 11.30 A.M. to 5 P.M.), proved sterile, like the dried slip from the vacuum tube exposed to uninterrupted sunlight for six hours.

It is thus abundantly clear that bright sunlight is lethal within six hours to *B. pyocyaneus* in the dried state, *in vacuo*, and that the resistance of the dried bacillus to this agency is not materially exalted, if it is increased at all, under the condition last stated.

The results show, in passing, that the view sometimes taken of the lethal effect of sunlight upon bacterial cultures, viz., that it is due to chemical decomposition of the medium on or in which the micro-organism is growing, is unnecessary to explain the result.

This being so, it was, perhaps, hardly necessary to test the lethal action of the short wave-lengthed ultra-violet rays upon the dried bacillus, except for the sake of confirming an obvious deduction by actual demonstration.

In the following experiment, the action of the ultra-violet rays was tested upon *B. pyocyaneus*, which had been dried *in vacuo*, but upon the slip after its removal from the vacuum tube, and in an atmosphere of nitrogen.

It may be noted here that the intense light generated by the apparatus is *per se* also lethal, but not within the same time. This admits of being demonstrated by intercepting the passage of the ultra-violet rays by means of mica.

May 26, 1911.—A set of slips were inoculated with a 24 hours' growth of *B. pyocyaneus* in peptone water. A slip was transferred to each of four tubes, which were then exhausted, etc., and kept for five days, connected with the bulb of charcoal surrounded with liquid air.

May 31.—One of the tubes was opened, and the slip removed and exposed in an atmosphere of nitrogen for 15 minutes to the action of the ultra-violet rays. The slip was then transferred to a tube of litmus dextrose broth, and incubated at 37° C. as usual. The object of carrying out the exposure in nitrogen is to prevent the formation of ozone from the atmospheric oxygen, which would *per se* be lethal.

A second vacuum tube was then opened, the slip removed, and exposed in the same way to the rays for 30 minutes, after which it was transferred to a tube of litmus dextrose broth. No growth occurred in either tube, proving that the action of the ultra-violet rays in an atmosphere of nitrogen had been lethal.

June 1.—Another of the same batch of vacuum tubes was opened, and the slip transferred to litmus dextrose broth. A growth of *B. pyocyaneus* had occurred within 24 hours.

The details of the technique employed, which were devised by Sir James Dewar, were as follows: The air admitted to the vacuum tube, after being sealed off, was air which had been dried and deprived of particulate material, by passing it through a **U**-tube containing cotton-wool, immersed in liquid air.

The slip was transferred, with the film uppermost, to a capsule of German silver, into which, near the bottom, a side tube was soldered, this being connected with leaden tubing to a Dewar vessel containing liquid nitrogen, from which the gas was regularly discharged by slow ebullition throughout the experiment.

The German silver capsule was fitted with a lid of quartz, and placed in a beaker of pounded ice. The lid of quartz was made to fit fairly tightly inside the upper part of the metal capsule, so that the nitrogen could only slowly escape from the chamber.

After two or three minutes to allow of the replacement of the air by the nitrogen, the ultra-violet rays were switched on. The distance from the horizontal quartz tube, whence the rays were emitted from the arc passing between two mercury poles, to the top of the capsule was about 2 inches.

May 26.—This observation was repeated in all details, upon slips of *Staphylococcus pyogenes aureus*, which had been dried *in vacuo* for the five preceding days, the connection with the bulb of charcoal, etc., being maintained throughout. Four tubes were sealed off, and on the same day two were opened, the slips removed, and subjected, with the prepared side upwards, to the rays, for 15 and 30 minutes respectively. After the exposure, each slip was transferred to a tube of litmus dextrose broth.

No growth took place in either tube.

June 1.—A control vacuum tube sealed off on May 26, after five days' connection with the bulb of charcoal and liquid air, was opened, and the slip transferred to a tube of litmus dextrose broth. Growth occurred within 24 hours.

So far as the possibility of interplanetary bacterial life is concerned, then, it is evident that bacteria in the fully dried state, if free in the interplanetary vacuum, would be killed by the solar light and ultra-violet rays.

And, as Sir James Dewar's experiments have demonstrated that the ultra-violet rays will kill undried bacteria whilst in the frozen condition, at the temperature of liquid air (−190° C.), there is little to support the hypothesis that the living protoplasm on the earth originally immigrated from interplanetary space in a free or unincluded condition—that free, particulate life has entered the earth's atmosphere as a result of light propulsion, from extra-mundane space.

An Improved Method for Opsonic Index Estimations, involving the Separation of Red and White Human Blood Corpuscles.

By Charles Russ, M.B. Lond.

(Communicated by Dr. A. D. Waller, F.R.S. Received February 21,—Read March 14, 1912.)

Opsonin is the name given by Sir A. E. Wright to the substance in human blood which mainly influences phagocytosis. In order to measure the opsonin, equal volumes of normal serum, of a bacterial emulsion, and of leucocytes are incubated to promote phagocytosis, and the number of bacteria visible in a random sample are counted in a stained film preparation. By repeating the experiment with a pathological serum, a ratio is obtained which is known as the opsonic index. The method, however, has been found inaccurate, and consequently fallen almost into disuse. Drs. Greenwood and White pointed out the error of random sampling and, after elaborate mathematical analysis, adjudged the "liability to error" on a single count to occasionally exceed 20 per cent. from the mean or true figure. Clearly, when the counts from two films are contrasted, the liability to error may be doubled.

This liability to error is due to the variation in content of different leucocytes in the same film ranging from 0 to 25 bacteria, and the resulting total in 50 leucocytes will vary according to the field in which the operator happens to make his count, *i.e.*, one rich in high or low contents.

In my work this error has been studied and largely reduced. The first idea was that the number of leucocytes in the mixture might vary in the successive sets of materials. To test this point I repeatedly estimated the opsonin of normal serum to staphylococci, and also counted the leucocytes per cubic millimetre in each opsonic mixture. The results are shown in Table A.

Table A.—Opsonic Index. (Old Method.)

Repeated estimation of the opsonin to *Staphylococcus aureus* in normal serum and simultaneous enumeration of the leucocytes per cubic millimetre in each opsonic mixture.

Series.	Leucocytes per cu. mm.	Opsonic count 50 leucocytes.	Error *above* average.	Error *below* average.	Mean value.	Blanks per 50 leucocytes.	Worst error *above* average.	Worst error *below* average.
			per cent.	per cent.			per cent.	per cent.
1	15 × 200	279	—	4	292	9	—	—
$\frac{26}{1}$	9 „	337	15	—		9	15	—
	14 „	285	—	2		7	—	—
	11 „	268	—	8		6	—	8
2	18 „	190	11	—	170	10	—	—
$\frac{16}{1}$	16 „	258	52	—		3	52	—
	19 „	118	—	30		20	—	—
	10 „	114	—	33		20	—	33
3	14 „	185	8	—	170	16	—	—
$\frac{23}{1}$	9 „	155	—	8		14	—	—
	6 „	213	25	—		13	25	—
	16 „	137	—	19		21	—	19
	36 „	180	5	—		13	—	—
	12 „	155	—	8		14	—	—
4	12 „	151	—	20	190	11	—	20
$\frac{15}{1}$	7 „	191	—	—		7	—	—
	25 „	191	—	—		8	—	—
	10 „	224	17	—		4	17	—
	Total............		133	132				

Average error above or below the mean value = 265/18 = 14·7 per cent.
Worst error *above*... = 52 „
Worst error *below*... = 33 „

The encircled numbers indicate the highest leucocyte content in each series and the lower figure = the number of times it occurred.

All the materials were identical in each series, and the opsonic estimations by the same operator, and the observations made at about half-hour intervals.

The following points are apparent from these experiments:—

(1) The leucocytes did vary in the series used for the opsonic estimations.

(2) No definite relationship was apparent to a high or low count from this leucocyte variation.

(3) The opsonic count often showed large variations from the mean value, *i.e.* a large experimental error.

(4) There appeared to be the all-important constancy of the opsonic figure when the numbers of blanks met during a 50 count was the same or nearly so.

This last feature suggested that this large content variation might be due to uneven access between leucocytes and bacteria. However, the outstanding fault of the process is this large variation in the leucocyte content, and its occurrence could only be due to differences of (1) *appetite* or (2) *opportunity*, or a combination of these factors.

(1) *Appetite.*—If the leucocytes have equally as good chances to pick up bacteria in the opsonic mixture, and yet show this variation, it must be presumed physiological, and there is no remedy.

(2) *Opportunity.*—It may be that all the leucocytes have similar appetites, but get very different opportunities to pick up bacteria, owing to their uneven distribution in the mixture.

A scrutiny of the materials used in the old method showed two important defects.

The Presence of Red Corpuscles.—Although white corpuscles only are concerned in the process, both red and white blood corpuscles are used; but, since a bacterial suspension not exceeding 500M per cubic centimetre is used, and washed blood corpuscles contain 5000M red and 10M white corpuscles per cubic centimetre, it is evident that for every leucocyte there are 50 bacteria provided, but surrounding this all-important leucocyte are 500 obstructing and useless red corpuscles. I therefore decided to abolish the red corpuscles, and this involved the *separation of red and white human blood corpuscles.*

The methods which were tried unsuccessfully included—Hæmolysis of the red corpuscles; agglutination by ferric chloride and filtration of the red groups; filtration of decalcified blood; sedimentation of decalcified blood after artificially raising the specific gravity.

However, Dr. Ponder's work on leucocytes furnished the nucleus of a successful method. He found that when blood is enclosed in a cell between two glass plates and incubated, there occurs a swarming of polynuclear leucocytes to the glass surfaces, to which they adhere firmly and appear remarkably distorted.

My first problem was to get these leucocytes off the glass. This was found to be effected by citrate saline solution 1·5 : 0·8 per cent., by disodium hydrogen phosphate, and by hypertonic salt solution, and also by serum. After obtaining a large number in a test-tube in the citrate solution (to make films), I found they were highly unstable osmotically and, when transferred to normal saline, the majority burst.

After experimenting with over 200 Ponder plates, I realised that the leucocytes could only be obtained in bulk if favourable chemical conditions were ascertained (since incubation aggravated the bursting). The following method succeeded in supplying a majority of polynuclear leucocytes (the lymphocytes do not appear on the plates) in good condition, which could be incubated for 15 minutes, as in the opsonic index process. The detailed method is as follows:—

Blood is shed into a rubber ring (cell) sandwiched between two glass plates. This cell is incubated for 20 minutes at 37·4° C., removed from incubation, the cell is opened, the clot and ring removed, the plates washed with 1·25-per-cent. saline, to free them from red corpuscles and serum. After wiping the ring margin clear of more red corpuscles and dried serum a few drops of cold NaCl (1·25 per cent.) are poured on the leucocyte-laden area of each plate. These are replaced on the metal shelf of the incubator for 15 minutes; when the plates are inspected the previously distorted and stretched out polynuclears will be seen under a low objective to have become almost spherical and loose from the plate. By means of a long glass rod the fluid and floating leucocytes are swept into a small tube and concentrated by the centrifuge at moderate speed. After syphoning off the supernatant fluid a very large number of human polynuclear leucocytes were obtained, which stand incubation with equal volumes of serum and the bacterial emulsion, the latter being made with 1·25-per-cent. NaCl instead of normal saline.

But before proceeding to test the opsonic process for the anticipated increased accuracy the second defect of opportunity in the old method had to be attended to. In the old method the opsonic mixture (serum, bacteria, and washed blood corpuscles) was incubated for 15 minutes, but even at the end of 10 minutes the bulk of the corpuscles had settled to the bottom of the glass pipette, the supernatant fluid being clear. Since equality of opportunity cannot exist when this is permitted this defect was remedied by keeping the mixture in slow rotary motion during incubation by means of the opsonic mill (fig. 1), and by this device no sedimentation occurs, but there is some degree of active mixing.

A mechanism to prevent sedimentation of the opsonic mixture was devised by Rosenow, 1906, and by Glynn and Cox, 1912.

The latter used a mechanism which rotated the pipette on its long axis in a horizontal plane, and their experiments showed no reduction of the error by its use when tested.

Only a small benefit is to be expected from such an improvement, but it is probably inappreciable when the entire experimental error may be large, as in the old method.

Moreover, their method of rotation is not ideal, for though sedimentation is prevented, there is no active mixing (from end to end of the pipette) induced by such a roller movement. In the device illustrated in fig. 1, not only does no settling occur, but experiments showed that the corpuscles pass up and down in the opsonic fluid during the changing positions of the pipette in the slowly moving wheel.

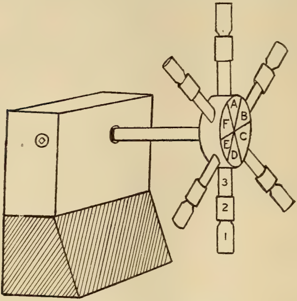


Fig. 1.—The Opsonic Mill.

This clockwork-driven instrument standing in the incubator rotates the opsonic mixture and prevents sedimentation of the corpuscles. Speed of rotation = 1 min. 45 secs. per revolution. (1) The shortened opsonic pipette. (2) Indiarubber collar. (3) Copper tube lined with fine copper wires. The brake is not illustrated.

After a few trial experiments with the new materials I proceeded to test the opsonic index of the same serum repeatedly, as in the experiments recorded (Table A), to ascertain whether the more even access and mixture of bacteria and leucocytes now improved the experimental error.

The results are shown in Table B.

Table B.—Opsonic Index. (New Method.)

Repeated estimation of the opsonin to *Staphylococcus aureus* in normal serum, using leucocytes only and the opsonic "mill."

The series number indicates a set of four or six observations made at a sitting with the same set of materials.

Series.	Opsonic count, 50 leucocytes.	Error *below* average.	Error *above* average.	Mean value.	Blanks, per 50 leucocytes.	Worst error *above* average.	Worst error *below* average.
		per cent.	per cent.			per cent.	per cent.
1. Very thin bacterial emulsion	61	16	—	72	27	—	16
	72	—	—		17	—	—
	76	—	5		21	—	—
$\frac{7}{1}$	79	—	9		19	—	—
	67	6	—		20	—	—
	84	—	15		14	15	—
2. Thin bacterial emulsion	117	—	13	103	13	13	—
	103	—	—		13	—	—
	114	—	11		12	—	—
$\frac{12}{2}$	87	15	—		11	—	15
	91	11	—		10	—	—
	108	—	4		18	—	—
3. Medium bacterial emulsion	130	6	—	139	6	—	6
	147	—	5		9	5	—
	135	2	—		11	—	—
$\frac{10}{1}$	144	—	3		8	—	—
4. Thick bacterial emulsion	160	10	—	178	4	—	10
	195	—	9		3	—	—
	197	—	10		6	—	—
$\frac{14}{1}$	163	8	—		7	10	—
Total		74	84				

Average error above or below the mean value = 158/20 = 7·9 per cent.
Worst error *above* the mean value............................ = 15 „
Worst error *below* the mean value............................ = 16 „

The results recorded in this table (B) show a marked reduction of the average and maximum error from the mean value, and this had occurred in spite of a fairly wide variation in the strength of the bacterial emulsion used. Believing that still higher accuracy might be obtained I undertook a further series of tests, matching the emulsion used in Experiment 3, Table B (which had been fixed by heat), as a standard. I also decided to count 100 leucocytes, 50 from each of the two films made from the mixture.

The results are shown in Table C, and show a still higher level of accuracy than those of Table B, though visual matching of the emulsions (to produce an average phagocytosis of three bacteria per leucocyte) was not very successful, though it can be assured in future by a preliminary count.

Table C.—Opsonic Index. (New Method.)

Repeated estimation of opsonin in normal serum to the *Staphylococcus aureus,* using leucocytes only, and the opsonic mill, and counting 100 leucocyte contents.

Series.	50 leucocytes, Film I.	50 leucocytes, Film II.	Opsonic count.	Mean value.	Error from mean value.		Blanks, per 100 leucocytes.	Maximum deviation from mean value.	
					+	−		+	−
					Per cent.	Per cent.		Per cent.	Per cent.
1	144	105	249	256	—	2	18	—	2
	127	144	271		6	—	18	6	—
$\frac{10}{2}$	107	146	253		—	1	16	—	—
	131	119	250		—	2	20	—	—
2	142	178	320	324	—	1	10	—	—
	170	159	329		1	—	15	—	—
$\frac{14}{1}$	150	160	310		—	3	16	—	3
	176	162	338		4	—	8	4	—
3	127	120	247	252	—	2	49	—	—
	128	103	231		—	8	40	—	8
$\frac{14}{1}$	144	113	257		2	—	32	—	—
	144	132	276		9	—	40	9	—
4	99	112	211	230	—	8	25	—	8
	114	144	258		12	—	19	12	—
$\frac{9}{4}$	121	109	230		—	—	23	—	—
	96	125	221		—	3	25	—	—
			Total		34	30			

Average error above or below the mean value = 64/16 = 4 per cent.
Worst error *above* .... = 12 „
Worst error *below* .... = 8 „

There was defective emulsification of the staphylococci in Series 3, evident in the "clumpy" films, but fortunately the entire results of the table can afford the handicap.

All the films of Tables B and C have been preserved.

Summary.

The improvements described have produced :—

(1) A striking reduction in the liability to error of opsonic estimations when repeatedly tested.

(2) The results recorded by the new method (Table C) showed a liability to error of about one quarter the magnitude of those recorded in Table A (old method), the experimental conditions being almost comparable.

(3) The enhanced accuracy is associated with a much reduced range of microbic content of the leucocytes (0 — 14).

(4) The improved results are attributable to the more even distribution of bacteria amongst the leucocytes (by the removal of the red corpuscles) and by its maintenance during incubation in the opsonic mill.

(5) No observations were made of any variations in opsonin in health or pathological states.

I am indebted to Dr. Cavendish Fletcher for his valuable assistance with this work, which unfortunately he had to abandon.

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On a New Method of Examining Normal and Diseased Tissues by Means of Intra-vitam *Staining.*

By Prof. Dr. Edwin Goldmann, University of Freiburg, Baden.

(Communicated by H. G. Plimmer, F.R.S. Received February 22,—
Read March 15, 1912.)

(1) *History of Method.*

The older methods of vital staining, such as the methyl-blue stain for nerve fibres, neutral-red stains for glandular cells, and others, were limited in their use to the differentiation of specific tissues. Ribbert was the first to attempt a vital stain of the whole body by intravenous injections of carmine solutions. The results achieved by this method in normal (Ribbert, Schlecht) and pathological tissues (Pari) were not certain enough to permit of its general use in histological practice.

A new departure in the field of vital staining resulted from the attempts to cure trypanosome and other infectious diseases of the blood through the agency of aniline dyes, such as trypan-red and trypan-blue. Bouffard, of the Pasteur Institute, investigated the cellular conditions following upon the injection of trypan-blue. Without previous knowledge of his work I undertook an extensive study of normal mice and rats, injected with trypan-, isamin-, and pyrrhol-blue solutions. The substance of this research was published in 1909 in a paper, "Äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der vitalen Färbung, Teil I."* Since then I have improved the method, searched for new staining media, and have finally applied the vital stain for purposes of comparative histological and pathological research.

(2) *Methods.*

A vital stain is achieved in an animal either by injecting the stain subcutaneously, intraperitoneally, or into the blood-vessels. Confining myself for the present to the two stains whose tissue reactions I have already published, I have now determined that in mice and rats trypan-blue acts equally well when injected under the skin as it does after introduction into the blood-vessels. But whereas 1 c.c. of a 1-per-cent. solution per 20 grm. of the animal's body-weight injected subcutaneously has no ill effect on the animal, no more than 0·5 c.c. of the same solution should be used for intravascular work. In the latter case coloration sets in speedily, increases up to the second day after injection, but rapidly fades after the fourth day, in any case quicker than when gradual absorption of the staining fluid takes place through the lymphatic channels.

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* 'Beiträge zur Klin. Chirurgie,' H. Laupp, Tübingen. In this paper, as well as in a more recent one, published in the same journal in March, 1912, plates are to be found which illustrate histological appearances referred to in this paper.

As for isamin-blue, the maximum dose for intravascular injection should not exceed 0·3 of a 1-per-cent. solution by a single drop, if its general toxic effect on the animal or the danger of widely-spread thrombosis is to be avoided. Where distinct and delicate granule staining is required the preference should be given to isamin-blue.

It is undoubtedly safest and best for histological study to inject the staining fluids subcutaneously. Injections of 1 c.c. of a 1-per-cent. solution per 20 grm. of the animal's body-weight may be repeated many times once a week. In some cases I have gone up to 15 injections, without doing any harm to the animal. Naturally, the vital coloration then becomes most intense and histological details very distinct. For bigger animals, such as the rabbit, dog, and ape, where larger and more frequent doses of the staining fluid are needed, intraperitoneal injections are preferable to subcutaneous, the dose being determined by the animal's weight. It is safe, as far as isamin- and trypan-blue are concerned, to abide by the standard, 1 c.c. of a 1-per-cent. solution per 20 grm. of the animal's weigh

Both isamin- and trypan-blue allow of fixation by means of a 10-per-cent. formalin solution (best applied from the beating heart of the anæsthetised animal), but it is only in tissues stained by trypan-blue that the ordinary processes of histological technique are applicable. Alcohol extracts isamin-blue also after lengthy fixation of tissues in formalin solution. But even after vital staining with trypan-blue it is advisable to carry along a small quantity of formalin in the dehydrating fluids. When these precautions are taken, durable histological specimens of vitally stained preparations may be obtained, which recall in every detail the conditions prevalent in life.

As a rule I use sections cut by the freezing microtome from specimens that have been fixed in a 10-per-cent. formalin solution for not less than 48 hours. The specimens are then transferred to a slide and allowed to dry until the section appears firmly fixed to the slide. Then the process of counter-staining, dehydration and mounting is rapidly performed. As to counter-staining, I have recently determined that, besides the alum-carmine stain I first advised, other methods likewise yield excellent results.

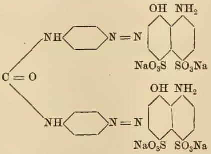
For studies of connective tissue reactions it is well to combine the vital stain with Pappenheim-Unna's pyrronin methyl-green solution, inasmuch as this combination permits of differentiating between the vitally-stained "pyrrhol cell," the basophile plasma cell and Ehrlich's "mast cell," distinguishable by the meta-chromatic orange colour which its granules display.

For purposes of leucocytic and lymphocytic analysis I have found the combination of the vital stain and Ehrlich's triacid mixture most useful. In this instance paraffin sections of tissues taken from animals which have been subjected to trypan-blue injections are necessary.

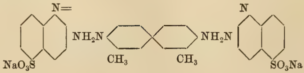
In my experiments on tuberculosis I have succeeded in retaining the vital stain even in sections which underwent the carbol-fuchsin staining and nitric acid differentiation for tubercle bacilli. Not only did we discover the bacilli, but we were able also to discriminate all the phases of disintegration, which the vitally-stained cell granules gradually underwent through the intracellular multiplication of the bacilli and their effect on cell structure. In this case also paraffin sections from trypan-blue animals are required.

The number of "vital stains" recently discovered by myself and by my pupil, Herr Schulemann, is rapidly increasing. Our chief object in the search for new stains is to discover the exact chemical radical which produces the vital reaction. We have as yet not come across a single "vital stain" which differs in its general behaviour towards the living organism from that of trypan- and isamin-blue. Hence, new proof has been furnished for my original contention, that in *intra-vitam* staining we are dealing with established chemical properties in the cell plasm and with an innate constitution of its structure. Amongst the new stains which are now engaging our attention I mention the following, appending a few remarks as to their application. All stains are procurable from Grübler and Co., Leipzig.

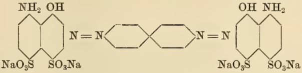
1. *Trypan-violet.*—It corresponds in all details of vital staining, application, fixation, etc., to trypan-blue. The formula is :—



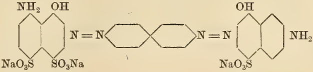
2. *Benzopurpurin B.*—



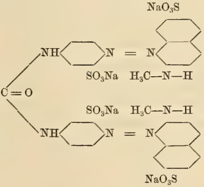
3. *Diamin-blue BB.*—It is an excellent *intra-vitam* stain. Herr Schulemann has found that 300 c.c. of a 1-per-cent. solution, injected into the peritoneal cavity of a dog weighing about 10 kgrm., produce within 24 hours a magnificent coloration of the animal. Its formula is as follows:—


NH₂ OH
OH NH₂
N=N
N=N
NaO₃S SO₃Na
NaO₃S SO₃Na

4. *Diamin-black BH.*—A most reliable vital stain, of which the formula is as follows:—

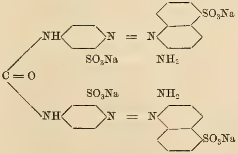

NH₂ OH
OH
N=N
N=N
NH₂
NaO₃S SO₃Na
NaO₃S

5. *Vital "Neu Rot."*—

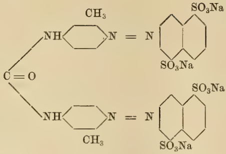

NaO₃S
NH
N = N
SO₃Na H₃C—N—H
C=O
SO₃Na H₃C—N—H
NH
N = N
NaO₃S

In mice 2 c.c. of a 1-per-cent. solution per 20 grm. of animal's body weight may be weekly injected. The effect is a brilliant scarlet stain of the animal. Unfortunately, this scarlet colour is transformed into a brownish red colour when the tissues are subject to formalin-fixation.

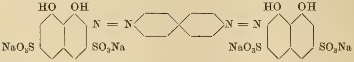
6. *Vital "Neu Orange."*—


SO₃Na
NH
N = N
SO₃Na NH₂
C=O
SO₃Na NH₂
NH
N = N
SO₃Na

7. *Vital "Neu Gelb."*—



8. *"Dianil Blau," R.*—



As to technique, I will merely add that a solution of these stains in Ringer's fluid is advisable for purposes of *intra-vitam* staining. It is well to begin experiments on animals whose toxic susceptibilities are not yet tested, by injecting a weaker dose than that which results from the formula for subcutaneous and intraperitoneal application—1 cm. of a 1-per-cent. solution for 20 grammes of animal's body weight.

Evidence gathered both in physiological and pathological fields seemed to point towards the fact that cell plasma, which accepts fat and lipoid stains, such as sudan, scarlet red, and Nile blue, also shows a marked tendency towards vital stains. But the exact relation between intracellular fat and lipoid substances, on the one hand, and bodies affected by the vital stain, on the other, was revealed by a careful analysis of the Pyrrhol cell (to be described in full below) during its transformation into the spindle cell of scar tissue and during the varied phases of its disintegration under the influence of bacillary necrosis. In both cases I discovered that when unimpaired, the pyrrhol cell accepts the vital stain only, whereas during the progress of its transformation or disintegration its affinity for the vital stain decreases and its powers of attracting the fat stains increase. Hence I am inclined to assume that, under normal conditions, fat or lipoid substances of the cell plasma unite with proteins and form loose compounds, liable to *intra-vitam* staining. Once this coherence is destroyed, the histo-chemical fat reaction becomes evident, whereas the vital stain is lost.

Similar relations between fat and albumen have already been demonstrated for the blood. Under normal circumstances, as long as fat is linked to albumen, its extraction by ether is rendered impossible by the "protective" influence of the proteid "radical." But during the period of lactation, under the influence of starvation and phosphorus poisoning, a decomposition of "blood fat" results, and it becomes soluble in ether. That the vital stain is not directly due to the presence of fat or lipoid bodies may also be inferred from the fact that I have hitherto failed to discover a fat or lipoid solvent for any of our vital stains.

(3) *Normal Tissues.*

I quote the following general facts from my first paper:—

"Throughout the whole of the animal's tissues the stain is embodied in granules of specific cells. Although the stain circulates in the blood, no blood cell accepts it, nor has it any effect on the cells of the vascular coats. In the skin the stain is discovered in the granules of the fixed connective tissue cells of the cutis, but chiefly in free round cells belonging to the lower layer of the subcutis. Here the cells aggregate in great numbers, and especially in spots where an irritation or lesion of the skin is produced by artificial means or by pathological processes. These cells which belong to the type of the 'histiogenic migratory cell' are by no means confined to the skin; they appear in every internal organ (with the one exception of the nervous system) and always in connection with interstitial fibrous tissue. We find them in muscles, tendons, in glands, but especially in serous membranes. The cells display besides marked chemotactic irritability and migratory powers phagocytic properties, which are most obvious in peritoneal lesions of every description. As the granules of their protoplasm eagerly absorb vital stains such as pyrrhol blue, I originally called them pyrrhol cells to emphasise their specific 'vital reaction' and to distinguish them from the various leucocytic and other migratory cells, which appear in the connective tissue."

In the course of my newest work I have discovered that the pyrrhol cell is to be found in great numbers in the subcutaneous tissue and the superficial muscular layers of the embryo towards the end of its development, and also under the skin and in superficial lymph glands of the new-born animal. Both in new-born and adult animals it is an inhabitant of the bone-marrow. It seems probable that the bone-marrow is concerned in the production of pyrrhol cells. My latest work, especially on the rat, has proved that the matrix of these cells is situated in the *tâches laiteuses* of the omentum and those of certain peritoneal ligaments, such as the ligamentum gastro-lienale and others. Only by means of the vital stain can we safely and surely distinguish the pyrrhol cell from its endothelial neighbour, the latter being refractory to the stain. The pyrrhol cell, when leaving the *tâche laiteuse*, spreads along the perivascular lymphatics into the wide area of the peritoneum, also reaches the peritoneal cavity, where even in its vitally stained condition it becomes the "macrophage" *par excellence*, absorbing not alone dead material, such as carmine, dust, etc., but also living tubercle bacilli, manifesting a predilection for those of the avian type.

The importance of these cells will be fully illustrated by our inquiries into pathological conditions, such as tuberculosis and malignant growths.

By means of the *intra-vitam* stain we can differentiate the "Kupffer" star cell in the liver, the reticulum cell of lymph glands, spleen, and bone-marrow, the interstitial cell of the testicle, the follicular cell in the maturing Graafian follicle, the cortex cell of the suprarenal, the epithelial cells of the choroid plexus, the epithelial lining of the convoluted tubes of the kidney. Most striking is the appearance of the placenta and its behaviour in relation to the rest of the body. When pregnancy occurs in the vitally stained animal, the blue colour disappears from its skin and is concentrated in the uterus, the latter forming a centre of attraction for the vital stain, and actually dispossessing the remaining tissues of their blue. In the uterus we find the blue chiefly in the free cells of the decidua serotina, but also in the cells of the reflexa during the period of its existence.

In the first days of pregnancy, during which the development of the mouse and rat embryo is slow and its growth is solely dependent upon exuded maternal blood, I have recently found that peritoneal pyrrhol cells migrate into the uterine wall, penetrate into the primitive placenta and cast off vitally stained granules, which are snatched up by fœtal cells in the way of nutritive material. Once the placenta has attained its maturity we discover the vital stain in the "giant cells," which form the boundary line between the maternal and fœtal part of the placenta. We also find it in those fœtal cells which constitute the only barrier between the maternal blood spaces and the endothelial lined capillaries of the fœtus. Finally, the vital stain effects a most striking specific differentiation in the granulated cells of the vitelline membrane. Notwithstanding the fact that the yolk membrane is deeply stained throughout its whole extent, and the placental fluid shows a faint bluish colour, the embryo remains perfectly colourless, the placenta and its appendages thus forming a kind of protective barrier against the passage of the stain from the maternal into the fœtal organs.

As to the later stages of embryonic growth my new histo-chemical studies have proved that exactly the same fœtal cells of the placenta, which so vigorously absorb the vital stain, store also glycogen, fat, and hæmoglobin temporarily, ere these substances pass into the fœtal circulation. In the light of my new work the importance of vital staining for the purposes of embryological research will become more apparent.

(4) *Diseased Tissues* (*Mice and Rats*).

(*a*) Healing of wounds.
(*b*) Trichinosis.
(*c*) Experimental tuberculosis.
(*d*) Toxic degeneration of the liver.
(*e*) Malignant growths.

(*a*) In the healing of wounds produced in the skin, liver, and kidney, the pyrrhol cell appears on the scene after the initial emigration of leucocytes from the dilated blood-vessels has taken place. The extravasated leucocyte shows glycogen and fat granules in its protoplasm. The pyrrhol cell phagocytes such leucocytes or incorporates either glycogen or fat granules derived from leucocytic degeneration. Eventually it stretches into the spindle cell of the young connective tissue, losing consecutively both its affinity for the vital and fat stains. In skin wounds the pyrrhol cell is derived from the subcutaneous tissue, whereas in liver and kidney wounds I have been able by means of the vital stain to demonstrate the migration of pyrrhol cells from the serous coat of the injured organ or from the peritoneal cavity into the wounded area.

(*b*) The activity of the pyrrhol cell is most prominently displayed in the surroundings of parasites, such as the trichina and other worms. In the case of the trichina the "pole cells" of the muscular "spindles" accept the vital stain. It appears that in wandering towards the trichina the pyrrhol cell passes through lymphatic glands, whose marginal sinuses and lymphatic spaces in general abound with vitally stained cells of the pyrrhol type. These cells first spread into the interstitial muscular tissue and thence penetrate the sarcolemma, arranging themselves on the outer surface of the encapsuled parasite. Purely mechanical conditions are responsible for the typical arrangement of the pole cells at the extremities of the "trichina spindle."

(*c*) *Tuberculosis.*—I have established a fundamental difference in the distribution of avian and bovine bacilli of tuberculosis, when grafted into the peritoneal cavity of the mouse. Hitherto, in all cases of spontaneous tuberculosis in the mouse, the "avian" bacillus has been found. Koch had already drawn our attention to the chronic course of tuberculosis in the mouse. And yet, when the mouse is subjected to an injection with bovine or human tuberculosis, either through the blood-vessels or the peritoneal cavity, the disease runs a comparatively rapid course, in many cases assuming a form of bacillary septicæmia or miliary tuberculosis of the lung. In accordance with these facts I am able to show that after peritoneal injection of bovine material, besides rapidly caseating tuberculosis of the peritoneum, the chief seat of trouble is the lung, whither the bacilli are carried by the blood stream after penetrating the portal vein and causing extensive tubercular thrombi throughout its larger branches. The liver and spleen contain merely microscopic lesions of inferior gravity, when compared with the large areas of tubercular necrosis in the lung. The pyrrhol cells of the peritoneum take no active part in this acute form of experimental tuberculosis.

An entirely different result follows the intraperitoneal injection of the avian bacillus. On macroscopic examination of the vitally stained animal, several weeks after inoculation of the virus, the peritoneum and intraperitoneal organs hardly show any trace of disease. All the more remarkable are the lesions revealed by the microscope. The omentum is full of blue patches, which to the naked eye wear the appearance of *tâches laiteuses.* By means of the specific stain for bacilli I was able to prove that these blue patches consisted entirely of pyrrhol cells, whose blue protoplasm was choked with myriads of bacilli. No trace of inflammation could be found in their immediate or more distant surroundings. Such aggregations of pyrrhol cells laden with bacilli were also discovered in the liver, spleen, mesenteric glands, and, in a smaller number, in the lung. In all these organs the tubercles had the vital stain and were thus easily distinguished by a low magnifying power. They lay in lymphatic spaces, in the liver surrounding the portal vessels, in the spleen arranged round the malpighian bodies. The blood-vessels were, with few exceptions in the liver, intact. No caseation occurred nor could small cell infiltration or giant cells be anywhere found in connection with these vitally stained tubercles of peritoneal origin.

A key to the whole process was afforded by the examination of animals at short intervals after the injection of the avian bacilli. The latter are quickly conveyed to the liver, where they are destroyed in great numbers by the Kupffer cells. Such as remain in the peritoneal cavity are phagocyted by the vitally stained pyrrhol cell. They multiply in those cells, which wander into the omentum, liver, spleen, and mesenteric glands, and eventually into the lung. As the bacilli increase, a most characteristic morphological change occurs in the cell. The granules of the protoplasm gradually disintegrate, the vital stain, which had originally been confined to the granule, now effects a diffuse stain of the whole cell. Eventually it can disappear entirely.

As this metamorphosis of the cell protoplasm proceeds, its biochemical reaction alters, inasmuch as in the place of a specific attraction for the vital stain, the protoplasm now shows an increased affinity for the fat stains. Fat first appears in the shape of tiny droplets. In the final stage, these droplets increase in size and eventually usurp the place of the cell body. And yet the cell continues to live, for even in this stage of excessive fat infiltration the nucleus accepts the nuclear stain and shows no signs of degeneration. Naturally, in the end, the cell succumbs. But, after death, even fat disappears from its necrosed body, whose shape still remains visible as a ghost in the tubercles undergoing necrosis.

We have thus established a fundamental difference in the distribution of bovine and avian tuberculosis, when injected into the peritoneal cavity of the mouse. In the bovine variety, metastasis occurs along the blood stream through the thrombosed portal veins; in the avian variety the dissemination is effected by the lymphatics.

What prospects these experiments on the *intra-vitam* staining of tubercles hold out to chemo-therapy hardly need be mentioned, since we now know that the germ-bearing cell is selectively affected by the vital stain.

(*d*) Toxic degeneration of the liver was produced by various poisons, such as phosphorus, cumarin, cocaine, and, above all things, by a substance first prepared by Ehrlich, called icterogen. The latter is an arsenic compound of Ehrlich's 606 series. On injection of a centigramme of a 1/5000 solution the mouse develops severe jaundice, followed by miliary bland necrosis of liver cells. Similar to cumarin, icterogen induces thrombosis in the smaller interlobular portal vessels, which explains the consecutive necrosis of liver cells. I have included these experiments in this paper merely to show that, wherever non-inflammatory necrosis in the liver occurs, its organisation is attempted by vitally stained pyrrhol cells, which leave the peritoneal cavity, migrate along the liver lymphatics towards the seat of trouble, and eventually assist in the repair of the damage.

(*e*) *Malignant Growths.*—In no case, to my knowledge, does the aggregation of blue-stained pyrrhol cells assume such extraordinary dimensions as in the instance of malignant growths placed under the skin. They swarm around the growing tumour and penetrate it along the endless blood channels which furrow its lobules. In the interior of the growth most of these cells succumb. I am still engaged in an inquiry into the relations existing between the growing tumours and these cells. Not wishing to forsake the sure ground of established facts, I merely state that the appearance of these blue-stained cells on the field of tumour grafts may be regarded as a specific local reaction induced by the tumour cell. When exempt from inflammatory agents, the tumour attracts no other migratory cell, but only the blue-stained pyrrhol cell. I am inclined to believe that these cells are the bearers of nutritive material for the growth. My new chemo-therapeutic experiments on mouse tumours by means of agents which damage the liver, such as icterogen and "Jod-phenyl arsensaures Natrium," have brought to light another important fact in connection with pyrrhol cells. They absorb the degeneration products of the liver cells, preferably the bile pigments, carrying them to the malignant growth. Hence the latter may wear both on its surface and in its interior a distinct yellow appearance, due to the aggregation of "jaundiced" pyrrhol cells. Since the tumour evidently suffers through the application of the above-mentioned substances, it does not seem improbable that the pyrrhol cell is also active in transporting substances to the tumour which impede, and in many cases stop, its growth.

Antelope and their Relation to Trypanosomiasis.

By Dr. H. L. Duke.

(Communicated by Sir J. R. Bradford, Sec. R.S. Received February 26,—Read March 28, 1912.)

[Plate 2.]

The flies on the Chagwe Lake shore are still capable of infecting monkeys with *Trypanosoma gambiense*. Four years and a-half have now elapsed since the Chagwe coast line was officially declared free of population, all villages destroyed within a zone of two miles bordering the lake, and their inhabitants removed inland. In spite of precautions, however, there is very little doubt that the islanders continued secretly to visit the mainland until they also were removed in 1909, and all the island villages destroyed. Since September, 1909, therefore, there have been no natives in the fly area except the recalcitrant few, who, at the risk of imprisonment, may from time to time return to their old haunts on the mainland or the islands. Instances of this have indeed occasionally occurred up to the time of writing. Apart from the captures made by the Government patrol, I have on several occasions had to report signs of recent native occupation within the prohibited area seen in the course of excursions from Mpumu.

In considering the question of the infectivity of the lake-shore flies, the possibility of infected natives being available as a food supply must still be considered, although this factor is apparently of small importance. These natives would most likely only venture forth at night time, and would be unlikely to approach the fly ground, which is visited almost daily by the boys from Kibanga. There remain the canoemen and fly-boys employed by the Laboratory, and in this connection it can only be said that, although frequently examined, they have never shown any sign of Sleeping Sickness either clinically or upon blood examination.

As all the mainland flies in the following experiments were caught upon a short stretch of shore to the west of the mouth of Buka Bay, which is visited several times weekly, it is extremely improbable that stray natives alone could account for the prolonged infection. The suspicion that either the antelope or reptiles which frequent the lake shore are acting as a reservoir for *T. gambiense* becomes increasingly probable the longer the wild flies remain infective. As to whether reptiles can serve as a reservoir, the difficulty of obtaining live crocodiles or water-lizards has, up to the present, prevented any satisfactory investigations being carried out on this point. That the buck are important in this connection was foreshadowed by results obtained in the laboratory experiments with these animals, some of which have proved capable of infecting laboratory-bred *Glossina palpalis* more than twelve months after their original infection with *T. gambiense.* If an infected buck took up its quarters in the forest behind the fly ground, it might account for the recent outburst of infectivity among the fly after a long period of relative quiescence. From prolonged observation, it would appear that bushbuck, at any rate, among the antelope frequenting the lake shore are very limited in their feeding range.

The objection that these animals would not, by virtue of their nocturnal habits, be exposed to the bites of the fly, may be dismissed at once. On several occasions I have seen bushbuck feeding at the water's edge as late as 8.30 A.M., long before which time the sun is powerful and the fly actively aggressive. Upon one occasion a herd of waterbuck was seen actually in the water at 11 A.M., in the full glare of the sun. Situtunga also, as will be seen later, are by no means so exempt from the attentions of the fly as the general impression of their habits would suggest.

Against the objection that the thick hair of an antelope would prevent a fly from piercing the skin over the greater part of the body, I may say that I have observed Stomoxys in numbers vigorously sucking blood on the haunches and sides of the body of a newly-killed bushbuck, regions where the hair is exceedingly thick.

As regards the possibility of the longevity of the fly affording a solution of the problem, I am unaware of any evidence of *G. palpalis* surviving for more than 227 days—an observation recorded by Dr. Kleine. With a view, then, to completing the chain of evidence incriminating the lake-shore antelope, the experiments given below were undertaken. Owing to the nature of the country, the expectations of a positive result from the mainland were extremely poor, as the places where buck can be shot are not necessarily places where fly abound. In the immediate vicinity of the fly ground the thick forest absolutely precludes shooting. The results obtained on Damba by Hammerton and Bateman in 1910, and again by Carpenter in May, 1911, showing that the flies on this uninhabited island are apparently maintaining their infectivity undiminished, suggested the scene for the experiments. The only species of antelope found upon the islands of Lake Victoria Nyanza is the Situtunga (*Tragelaphus spekei*). Since the removal of the natives from Damba, these animals have increased greatly in numbers, and at present their tracks may be seen at almost any suitable point on the shore of the island. As will be seen below, events justified the experiment and revealed the parallel in nature to the infections induced in the laboratory.

These investigations include, in addition to antelope, all other available vertebrates whose habits bring them into contact with the fly. In every case where possible the citrated blood was injected into a monkey and a goat and smears prepared. On rare occasions fresh preparations were examined. Certain mammalian trypanosomes other than *T. gambiense* were obtained in the experimental goats, and these are also discussed below.

The following is a brief summary of the experiments on the infectivity of the wild flies for *T. gambiense*, carried out during the period April, 1911, to January, 1912, covered by the paper:—

Table I.

Date.	Experiment.	No. of flies used.	Result.	Source of flies.
1911.				
Apr. 3—May 10	4	6144	+	Kibanga.
May 15—28	56	1050	+	,,
June 7—July 18	113	5385	+	,,
July 31—Aug. 31	234	6360	—	,,
Sept. 10—22	386	3310	—	Lwagi Island.
,, 10—22	387	3250	—	,,
Oct. 11—Dec. 5	459	6150	—	Kibanga.
Dec. 11—27	563	3190	+	,,

Remarks.—Four and a half years after the removal of the natives from the Chagwe coast line, and two years after the final clearing of the neighbouring islands, there is a percentage of 0·014 infective flies in a total of 28,279 caught at Buka Point, assuming that there is only one infective fly to each positive experiment. On Lwagi Island, where there are no antelope, it was impossible to obtain a sufficient number of fly to constitute evidence on the subject of *T. gambiense*. It will, however, be seen below that there are some grounds for believing that the negative results obtained with Lwagi flies are due to the absence of antelope from this island.

The actual injection experiments will now be considered. Each species of antelope is dealt with in a separate table, the remaining animals being grouped together. A blank space occurring in any column of the tables indicates that there is nothing to record under that heading.

Table II.—Waterbuck.

Date.	Expt. No.	Locality.	Microscopical examination.		Blood injection.				
						Monkey.		Goat.	
			Result.	No. of films.	Time after death of animal.	Quantity.	Result.	Quantity.	Result.
					hrs.	c.c.		c.c.	
21.4.11 ...	15	Enfufu ...			2	3	—	5	—
21.4.11 ...	15	„ ...			2	3	—	5	—
28.4.11 ...	26	Kyetume			3	3	—		
24.8.11 ...	324	Kibanga...	—	4	3	3	—	8	—
2.12.11 ...	544	„ ...			1½	3	—	6	—

Remarks.—With the exception of Expt. 26, all these antelope were shot within the prohibited area and may be considered as more or less frequent visitors to the lake shore.

Table III.—Bushbuck.

Date.	Expt. No.	Locality.	Microscopical examination.		Blood injection.				
						Monkey.		Goat.	
			Result.	No. of films.	Time of injection after death of animal.	Quantity.	Result.	Quantity.	Result.
					hrs.	c.c.		c.c.	
30.4.11 ...	28	Bugala	—	3	4½	3	—	5	—
30.6.11 ...	183	Kibanga......	—	6					
30.6.11 ...	185	„	*T. uniforme* + + +						
6.8.11 ...	249	Buwera	—	3	1½	4	—	10	—
13.8.11 ...	293	„	—	3	2	4	—	10	—
20.8.11 ...	312	Ebyoba			1	3½	—	10	—
2.9.11 ...	353	Kibanga......	—	2	1	3	—	8	—
27.9.11 ...	396	Kivuvu	—	8		3	—		
19.9.11 ...	405	Ebyoba	—	3					
5.10.11 ...	435	Namusenyu	—	3	1½	3	—	10	—
20.9.11 ...	466	Ebyoba	—	2	1	3			
19.10.11	472	Namusenyu	—	2	1½	3	—	5	—
21.10.11	479	Ebyoba	—	2	1			6	—
22.10.11	481	„	—	2	1½			5	—
22.10.11	482	„	—	2	1			3	—

Remarks.—With the exception of Expt. 396, all these buck were shot within a few hundred yards of the water's edge, and some actually on the lake shore. In only one case were trypanosomes discovered, *i.e.* Expt. 185, in whose blood an organism answering morphologically to *T. uniforme* was present in large numbers.

Table IV.—Hippopotamus, etc.

Animal.	Date.	Expt. No.	Locality.	Microscopical examination.		Blood injection.				
				Result.	No. of films.	Time after death of animal.	Monkey.		Goat.	
							Quantity.	Result.	Quantity.	Result.
						hrs.	c.c.		c.c.	
Hippo ...	23.4.11	17	Damba	—	2	2	3	—	5	—
„ ...	4.6.11	106	Namusenyu ...	—	2	3	2½	—	5	—
„ ...	11.6.11	132	Kibanga	—	2	2	2½	—	5	—
„ ...	10.9.11	469	Senkua Island	—	2	3	3	—	10	—
„ ...	11.9.11	470	Lwagi Island...	—	2	1			10	—
„ ...	19.9.11	404	Ebyoba	—	2	3½			6	—
„ ...	19.9.11	471	„	—	2	4	4	—		
„ ...	20.9.11	408	Senkua Island	—	2	7			6	—
„ ...	24.9.11	409	Damba Island	—	2	3½	3	—		
„ ...	24.9.11	406	„	—	2	3½	3	—		
„ ...	25.10.11	498	Kibanga			1½	4	—		
„ ...	4.11.11	547	Damba	—	2	3½	3	—		
Buffalo	4.5.11	37	Near Kyetume	—	4	1½	3	—	5	—
„	4.5.11	38	„	—	4	1½	3	—	5	—
„	11.5.11	49	Kibanga	—	4	4	3	—	5	—
„	1.6.11	99	Near Kyetume	—	4	1	2½	—	5	—
„	29.6.11	180	„	—	4	1	2½	—	5	—
„	31.10.11	499	Wankobe	—	2	1½	3	—	6	—
Wild pig	1.4.11	3	Kibanga	—	2	1	3	—	5	—
Otter ...	4.6.11	109	Namusenyu ...	—	4	2	2½	—	3	—

Remarks.—Expt. 499 was shot far inland. Expts. 37, 38, 99, and 180 were shot on the borders of the prohibited area. It is, however, probable that the herds, in the course of their wanderings, reach the lake shore from time to time, so that they may be considered in the present discussion. All the other animals in Table IV were shot along the lake edge.

On numerous occasions trypanosomes have been studied in the blood of Situtunga 173 and 356 (see Table V). These agree with *T. uniforme* as regards morphology and movement. Repeated attempts to subinoculate from both these antelope into sheep and goats have, however, up to the present failed. This failure to infect goats with the blood of game known to contain trypanosomes morphologically identical with *T. uniforme* has been already reported by Fraser and myself.

Experiments on these two antelope conducted with laboratory-bred *G. palpalis* show that only the proboscis is infected, the hypopharynx invariably containing a small number of free trypanosomes. This is another point of agreement with *T. uniforme*, which would thus appear the commonest antelope trypanosome of the Mpumu neighbourhood.

The organism referred to above as *T. ingens* in Experiment 173 has only been observed in fresh preparations. It is a large trypanosome with a constant, slow, rippling motion and a very wide undulating membrane. The

Table V.—Situtunga.

Date.	Expt. No.	Locality.	Microscopical examination.		Blood injection.				
						Monkey.		Goat.	
			Result.	No. of films.	Time of injection after death of animal.	Quantity.	Result.	Quantity.	Result.
31.7.11	173	Kibanga	*T. uniforme* + *T. ingens* +	?	Immediate	Several experiments	—	Several experiments.	—
30.6.11	551	,,	*T. ingens* +	4					
10.8.11	284	,,	—	6					
16.8.11	302	,,	—	3	3 hrs.	3 c.c.	—	8	—
22.8.11	317	,,	—	5	1 hr.	3½ ,,	—	6	—
28.8.11	332	,,	—	3	1 hr.	3 ,,	—	8	—
2.9.11	356	,,	*T. uniforme* +	?	Immediate	4 ,,	—	Several experiments	—
25.9.11	402	Damba Isle	—	3	2 hrs.	3 ,,	*T. gamb.* +		
25.9.11	403	,,	—	3	1 hr.	4 ,,			
5.11.11	509	,,	—	4	1 hr.	4 ,,	*T. gamb.* +	10	*T. vivax* + *T. uniforme* + *T. gamb.* +
6.11.11	510	,,	*T. uniforme* + fresh and stained		1 hr.	3 ,,		8	

Remarks.—Situtunga Expts. 173 and 356 are still alive at the laboratory.

T. ingens of Experiment 551 was seen in stained preparations and answered to the description given by the 1908–10 Commission for this trypanosome.

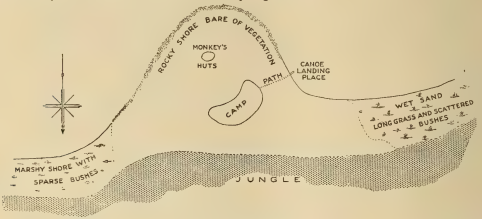
The reasons for the diagnosis of *T. gambiense* in the above table will be given below. It may be mentioned that Situtunga 173 has developed *T. gambiense* in its blood after being fed upon for five days by flies infected with this trypanosome.

The obtaining of *T. gambiense* from Experiments 402–3 and 509–10 are, of course, by far the most important result so far obtained during the examination of the lake-shore game. It is, therefore, necessary to consider fully all possible sources of fallacy in these experiments and to exclude them as far as possible. Upon this question Dr. Carpenter, at whose camp on Damba Island I made my headquarters, has kindly expressed his views as follows:—

The "Situtunga" Experiment.

"On considering this important result a possible source of fallacy at once suggests itself, namely, that as the monkey was infected whilst on Damba Island, the trypanosomes may have come from a glossina, for I have shown in a 'Progress Report' that the Glossinæ on Damba Island are still capable of infecting a monkey.

"This objection, formidable as it is on paper, is not so when the local conditions are thoroughly appreciated. In the first place, that part of the coast line where is the little promontory on which my camp has been since March is not sufficiently shaded for Glossinæ, which are only present in very small quantity. Moreover, the whole locality round the camp has been very completely cleared, and the edge of the jungle at the base of the promontory, in which there are few flies, is some 50 yards away from the nearest hut, and 100 yards at least from the monkey's quarters.


BARE OF VEGETATION
ROCKY SHORE
MONKEY'S HUTS
CANOE LANDING PLACE
PATH
CAMP
WET SAND AND SCATTERED LONG GRASS AND BUSHES
MARSHY SHORE WITH SPARSE BUSHES
JUNGLE

"Also, if an occasional fly should accompany the canoe back from that part of the shore where flies are very numerous (nearly two miles away to the south-west) it would not be carried on to the monkey's quarters, but would be intercepted by the boys' huts. As a matter of fact it is an exceptional occurrence to see any Glossinæ in the cleared area, and those that are occasionally seen are probably the bred flies which have escaped during manipulations, and these very quickly leave the locality.

"It might be suggested that one of these may have bitten an infected canoeman or fly-boy, and, subsequently, the experimental monkey; but I think it must be an exceptional occurrence for a monkey to be bitten by a glossina. A tame monkey which has been with me on the island since April, and *has never become infected*, is immediately aroused to alertness, even when half asleep, by the buzz of a glossina, and when one of my bred flies accidentally escapes the monkey is all eagerness to catch it, whereas an ordinary fly attracts little attention.

"Moreover, I have no reason to suppose that any of the natives with me on the island are potential sources of *T. gambiense*, and blood from all the fly-boys (who are most exposed to infection) has been injected into a monkey with negative result.

"(Signed) G. D. H. Carpenter,
"Damba Island, November, 1911."

It may in addition be pointed out in the case of Experiments 509–510 that both monkey and goat developed *T. gambiense*—confirmed in the latter by sub-inoculation into a monkey as well as in stained preparations; moreover, the goat was tethered in the camp some distance from the monkeys' boxes.

Since May, 1911, Dr. Carpenter has always had two monkeys in residence in the boxes left by Hammerton and Bateman, and though examined from time to time he has never had a case of spontaneous infection by stray flies. The following is a brief history of the animals which serve as a control to my experiments on Damba Island:—

Monkey A.—Arrived Damba, May 1, 1911, and was immediately used to test infectivity of wild Damba flies. Became infected on May 15, and was immediately shot.

Monkey B (into which the blood of Situtunga 402–3 was injected).—Arrived Damba, May 25, 1911, and between then and September 25, when the injections were made, a period of four months, was examined on 19 occasions, with negative results. On October 2 was negative, and on October 3 Dr. Carpenter first discovered trypanosomes in its blood.

Monkey C.—Arrived Damba, May 29, 1911, and from that date was used by Dr. Carpenter to test the infectivity of wild *G. palpalis* from the neighbourhood of Damba. It was examined on 36 occasions with no result during the ensuing five months. On November 6 it became infected, having by this time been fed upon by several thousands of wild fly.

Monkey D.—Tame monkey kept by Dr. Carpenter, and exposed to all the risks run by the experimental monkeys. Arrived Damba, March, 1911. In perfect health up till October 9, 1911, when, at my request, it was first examined. Since that date has been examined weekly, and has never shown trypanosomes, and is in perfect health.

Monkey E (into which blood of Situtunga 509–10 was injected).—Arrived Damba, October 8, 1911, having previously been examined regularly at Mpumu with negative results. Examined daily from November 3 to November 17 inclusive, with negative results. On November 5 and 6 received blood of Situtunga 509–10, respectively. On November 18 trypanosomes first appeared in its blood.

Monkey F.—Arrived on Damba, November 3, 1911, in perfect health. On November 4 injected with the blood of hippo, Experiment 547 (*cp.* Table IV). Between November 3 and December 12 examined on 21 occasions, with negative results. At present in excellent health.

Finally, it may be pointed out that the incubation period in the case of all three positive experiments was in keeping with previous experience. Thus

Monkey 401 first showed trypanosomes on the eighth day after inoculation. Monkey 511 showed first 13 days after the first injection, and Goat 512 developed *T. uniforme* 10 days, and *T. gambiense* and *T. vivax* 15 days after inoculation. With reference to the prolonged incubation period in the case of Monkey 511, it may be noted that, in the only case where an inoculation from the laboratory antelope has recently proved positive, the incubation period in the monkey before trypanosomes appeared was 18 days.

A consideration of the conditions on Damba Island affords a vivid picture—a vicious circle in constant operation, only awaiting the reappearance of the natives to reproduce the recent epidemic. With the exception of papyrus areas, practically the whole shore line of the island is to a greater or less extent fly-ridden, one portion being occupied by the famous fly-beach, from which for several years some thousands of pupæ have been brought monthly to Mpumu. In his recent report to the Royal Society, Carpenter describes an infection of a monkey with 885 flies caught in May, 1911, on this pupa ground. The situtunga are multiplying rapidly in the absence of the natives, and are far less restricted in their range than formerly. The common conception regarding the habits of this antelope, as being a creature spending its days in the papyrus swamps where no fly are found, and only visiting dry land at night to feed, does not apply to Damba. During the short time I spent on the island, I frequently saw situtunga from the canoe at the water's edge both morning and evening. As late as 9 A.M. I have seen them feeding among the ambatch stems in places where fly were numerous, and where it required constant vigilance to avoid being bitten. On one occasion I observed a female on the actual pupa-beach at 8.30 A.M. in the full glare of the sun. There is also a long extent of shore, where, owing to comparatively recent sinking of the lake level, there is now a strip of grass and bushes some 40–50 yards wide, dotted here and there with ambatch trees, between the forest and the water's edge. This is a favourite haunt of the situtunga, and tsetse are present throughout its extent. There is therefore every natural facility for that frequent interchange of infection between fly and buck which constitutes a true reservoir.

On the neighbouring coast of the mainland, the fly are probably brought into far less frequent contact with antelope. The stretches of shore where fly are numerous are few and far between, and the number of antelope which actually visit any given fly area would be small. On the mainland also, the situtunga would probably only be available to the fly residing in the immediate neighbourhood of the papyrus, and thus the most typically lake-shore antelope would be denied to the fly where the latter are most numerous.

Finally, the following table may be considered as supplying additional evidence on the part played by antelope as a trypanosome reservoir :—

Table VI.

Date.	Expt. No.	No. of flies used.	Result.	Source of fly.	Experimental animal.
1911.					
May 16—24.........	58	1100	*T. gambiense* + *T. uniforme* +	Kibanga	Goat.
July 24—Aug. 2...	223	1220	*T. uniforme* +	"	"
Sept. 27—Oct. 4 ...	400	1560	*T. uniforme* + *T. vivax* +	"	"
" 9—22.........	384	4258	—	Lwagi Island ...	"

Remarks.—It will be seen from this table that in the first three experiments, comprising in all 3880 mainland flies caught at Buka Point, *T. uniforme*, with or without *T. vivax*, appeared on all three occasions. A total of 4258 flies caught on Lwagi Island, where there are no antelope, but only hippo, birds, and reptiles, failed to produce either of these trypanosomes.

Diagnosis of the Trypanosome Obtained in Monkeys 401 *and* 511.

There seems no reason to doubt that the trypanosome obtained in monkeys from the blood of Situtunga 402–3 and 509–10 is identical with the species obtained by Hammerton and Bateman from wild Damba flies in May–June, 1910, and again in May, 1911, by Dr. Carpenter. The Damba natives suffered severely during the epidemic, as the conditions on the island ensured constant exposure to the bites of the fly. These fly are still infective to monkeys, and the trypanosome carried by them answers to *T. gambiense* morphologically, and as regards the disease in monkeys.

In the face of such facts a more comprehensive examination seemed hardly necessary. In the present instance, however, the importance of the issue at stake makes a careful investigation imperative.

Of the trypanosomes previously described from Uganda *T. brucei* is the only one which claims attention. Other species as *T. pecaudi* and *T.* "*dimorphon*" are also suggested by the morphology. The fact that *T. brucei* is supposed to be non-pathogenic to man emphasises the importance of excluding this species in diagnosing a trypanosome derived from wild antelope.

Behaviour of the Damba Trypanosome in Glossina palpalis.

In an experiment comprising 101 clean laboratory-bred *G. palpalis* fed upon Monkey 401, whose blood contained the Damba trypanosome, two infected flies were obtained on the 17th and 57th days respectively.

The clean monkey upon which they were fed developed trypanosomes answering to *T. gambiense*. The 57th day fly showed a typical *T. gambiense*

Table VII.

Origin of trypanosome.	Experiment No.	Microns.																			Average.
		17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
Situtunga 402–3………	401, monkey		2	2	1	4		2	1			3	1	1		2	1				24·4
,, ,, ………	477, rat		2		2		1	5			3	3	1	1	1	1					24·3
,, ,, ………	504, monkey			2	1	1			1	2	2	2	1	2				1	3	2	27·4
,, ,, ………	543, ,,	2	1	1		2	1	2	1	1	1	2	2	4							23·7
,, ,, ………	571, rat		1		3	5	2				1										20·1
,, ,, ………	573, dog				1		2	5	1	2	1	3		1	1	1	1	1			25·6
Situtunga 509–10 ……	(1) 511, monkey			1	3	3	5	3	1	1	1	2									22·35
,, ,, ……	(2) 511, ,,			2		5	6	2	2	1					1						22·0
,, ,, ……	525, ,,			2	1	2	1	4	1	4	2	2	1								23·5
,, ,, ……	574, dog			1	1	2	1	3	4	3	1	1	3								23·9
Totals …………	…………………	2		19	13	24	19	26	12	14	12	18	9	9	3	4	2	2	3	2	
Percentages …	…………………	1	3·5	9·5	6·5	12	9·5	13	6	7	6	9	4·5	4·5	1·5	2	1	1	1·5	1	

infection of gut, proventriculus and salivary glands, the proboscis being negative. This latter fact would point to the exclusion of *T. pecaudi* and *T. dimorphon* from the differential diagnosis. A second experiment, in which 51 laboratory-bred flies were used, proved negative.

An experiment comprising 79 laboratory-bred *G. palpalis* fed upon Monkey 511 gave one infected fly on the 42nd day. The gut only contained trypanosomes. The clean monkey upon which these flies were fed ran away without having ever shown trypanosomes.

Morphology.—Dimorphism marked, the extremes 35 μ and 17 μ. The long or short type may predominate from day to day, but intermediate forms are always present. Forms over 31 μ in length are very uncommon; the longest examples were met with in Experiment 504, a monkey infected from Experiment 401, through laboratory-bred flies.

Table VII on opposite page shows the distribution of length in 10 experimental animals.

The Protoplasm is devoid of granules in all preparations examined from goats and monkeys. In both the rats examined granules were present in numbers both behind and in front of the nucleus.

Table VIII.—Animal Reactions.

Animal.	Expt. No.	Source of virus.	Incubation.	Duration.	Remarks.
			days.	days.	
Monkey	401	Situtunga 402–3	8		Alive after 124 days; shows considerable emaciation; no lethargy.
„	511	„ 509–10	12–13		Alive after 82 days; no obvious symptoms.
„	504	Flies from 401	?		Alive after 75 days; in good health.
„	525	Goat 512	10		Alive after 70 days; in good health
„	543	Calf 478	10		Alive after 54 days; in good health.
„	575	Goat 579	6		Alive after 42 days; in good health.
Rat, white...	477	Monkey 401	5	83	
„ ...	571	„	5		Alive after 42 days; slight emaciation; lively.
Guinea-pig	458	„	10	61	
„	572	Rat 477	20		Alive after 42 days; apparently in excellent health.
Goat	512	Situtunga 509–10	15 (?)		Alive, and apparently in good health, after 82 days.
„	579	Monkey 401	?		Never showed trypanosomes, proved by sub-inoculation into monkey. Alive after 90 days; well.
Dog............	573	„	12		Alive after 42 days; in good condition.
„	574	Goat 512	14		In very poor condition at time of inoculation and during the experiment.
Calf	478	Monkey 401	?		Proved by sub-inoculation into monkey. Trypanosomes seen on one occasion 51 days after inoculation. No obvious symptoms after 99 days.

A free portion is always discernible in the flagellum, though, in the case of the stumpy forms, it is often extremely short.

Movement.—No marked translation; remains actively wriggling in one spot, making occasional short gliding excursions about the field. Often the side-to-side movements are of a jerky character.

These experiments though few in number afford valuable evidence in favour of *T. gambiense* to the exclusion of *T. brucei.* The sub-chronic type of the disease in rats is perhaps the most striking point in the above table.

Action of Human Serum.—The assumption that *T. brucei* is non-pathogenic to man rests chiefly on the susceptibility of this trypanosome to human serum. Laveran and Mesnil,* in describing the action of this serum on animals infected with *T. brucei*, confine their attention chiefly to rats. The following experiments in which animals suffering from the Damba trypanosome were treated with human serum may now be considered:—

Animal.	Ultimate source of virus.	Quantity of human serum inoculated.	Native supplying serum.	Condition of blood at time of inoculation.	Day after inoculation.									
					1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
		c.c.												
Monkey 401	Situtunga 402–3	3½	B.	+ + +	+ 1 seen	—	—	+	+	+ +				
„ 511	„ 509–10	3½	Y.	+ +	—	—	—	—	—	—	+	+	+ +	
„ 391	Human strain ...	3	Y.	+ +	+ 1 seen	—	—	—	—	—	—	+ 1 seen	+	+ +
Rat 477 ...	Situtunga 402–3	1	Y.	+ + +	+ + +	+ +	+ +	+ +	+ +	+ + +				
„ 576 ...	„	1	Y.	+ +	+ +	+ +	+	+ +	+ + +					
Monkey 563	Wild lake flies ...	3½	Y.	+ +	—	—	—	—	+	+	+ +			

Remarks.—It will be seen that in every case where the human serum was injected into a monkey the trypanosomes disappeared from the circulation for a longer or shorter period. In the case of the rats this did not occur. It would thus appear that there is some condition present in monkeys which causes them to react to human serum, this condition not being present in rats. Experiments conducted *in vitro* on the same lines appear to confirm this conclusion. The negative results obtained with Rats 477 and 576, together with the positive result obtained with the human strain in Monkey 391, neutralises what at first appeared evidence in favour of *T. brucei.*

As regards the diagnosis of the three species of trypanosomes described in Goat 512 (Table V), the following methods were employed:—

(1) Examination of fresh blood preparations.

By this means *T. uniforme* was identified in the blood of Situtunga 510, and in the case of Goat 512 all three species were recognisable.

(2) Examination of stained films.

T. uniforme identified in blood of Situtunga 510.

maxim., 19·00 μ; minim., 14·5 μ; average, 15·9 μ (dry fixation).

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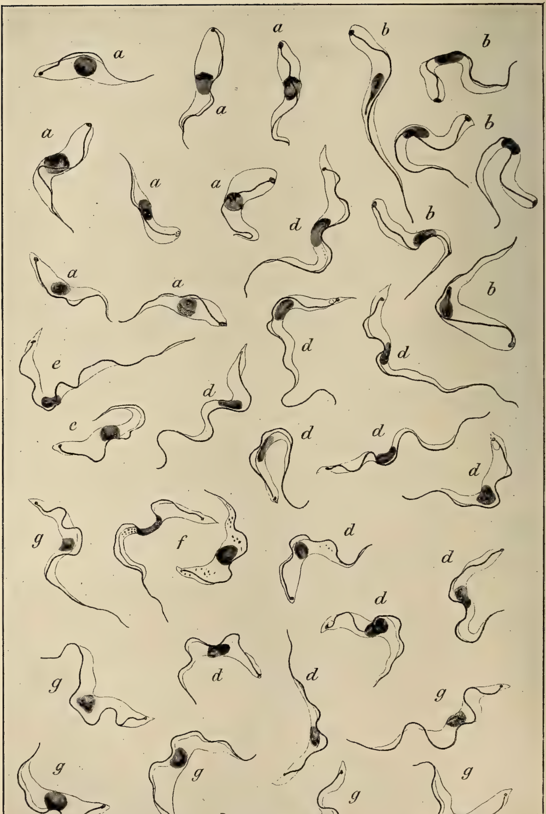
* Laveran and Mesnil, 'Trypanosomes et Trypanosomiasis,' 1904.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T. vivax in Goat 512.

maxim., 27·0 μ; minim., 20 μ; average, 23·9 μ (osmic acid fixation).

T. gambiense in Goat 512.

T. vivax and *T. gambiense* in centrifugalised blood of Goat 512.

(3) Sub-inoculation.

The blood of Goat 512 inoculated into Monkey 525 produced *T. gambiense* in the blood of the monkey.

Conclusions.

(1) That the continued infectivity of the wild *G. palpalis* on Damba Island to monkeys may be explained by the fact that the situtunga on that island are acting as a reservoir to *T. gambiense*.

(2) That the continued infectivity of the mainland flies to *T. gambiense* may probably be explained on a similar hypothesis, including possibly the other species of antelope frequenting the lake shore, *i.e.* waterbuck, bushbuck, duiker and reedbuck.

(3) That no positive evidence can be adduced from the above experiments to show that hippopotamus can serve as a reservoir.

(4) That the continued infectivity of the lake-shore flies to *T. vivax* and *T. uniforme* is also due to the antelope which serve as a reservoir for these trypanosomes.

DESCRIPTION OF PLATE.

(All drawn at magnification of 2000 diameters.)

a. T. uniforme from blood of Situtunga 511. Fixed by *drying only* followed by absolute alcohol.

b. T. vivax from blood of Goat 512. Fixed with osmic acid followed by absolute alcohol.

d. T. gambiense from blood of Monkey 401, which was infected from Situtunga 402–403. Fixed osmic acid followed by absolute alcohol.

e. T. gambiense from blood of Monkey 504, infected by laboratory-bred glossinæ from Monkey 401. Fixed osmic acid followed by absolute alcohol.

f. T. gambiense from blood of Rat 477, infected from Monkey 401. Fixed osmic acid and absolute alcohol.

g. T. gambiense from blood of Monkey 511, infected from Situtunga 509–510. Fixed osmic acid and absolute alcohol.

On the Presence of Radium in Some Carcinomatous Tumours.

By W. S. Lazarus-Barlow, M.D., F.R.C.P.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received February 14,—Read March 14, 1912.)

In another place* I brought forward evidence that acceleration of leak occurs when carcinoma tissue, after extraction with acetone or with ether, and subsequently with water, is introduced within an electroscope. The results have been criticised in respect of the smallness of the differences observed, and of their possible explanation by variations in the capacity of the electroscope occasioned by introducing the various substances within it. To meet the last criticism, an electroscope of constant small capacity was designed, which essentially consists of a closed metal box, divided horizontally by a wire grating. The upper part of the box contains the insulated gold leaf, and the various substances can be introduced into the lower part of the instrument through a well-fitting door.

Various non-malignant and malignant tissues, after extraction and in a dry, finely divided state, were then examined as to their possible influence on the leak. The majority of these were without effect outside the range of experimental error, but certain of them afforded evidence of acceleration. Specimens from both classes were then weighed, boiled with 10 c.c. hydrochloric acid and distilled water in a flask with side-piece, sealed, and set aside for four weeks. At the end of that time, the gas given off on vigorous boiling for five minutes was passed into an emanation electroscope in the usual manner and the leak determined.

The results of the examinations in the constant capacity electroscope are given on the opposite page.

A glance at the following table confirms the previous conclusion as regards acceleration, and in certain instances the acceleration is marked. In order to test the point further, Nos. 437, 440, 697C, 697G, 793, 147C, and 791 were examined for emanation, together with a sample of the HCl used for solution. Examination of the acetone, and of the ether used for extraction, was unnecessary, owing to the wide variation of the results obtained, in spite of the fact that one or other of these fluids was used in every case. Nevertheless, all reagents used were tested and found to be radium-free. None of the patients in whose cases radium was found had, during life, been treated with radium in any way or form, and care was taken to avoid contamination with radium during the manipulations.

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* 'Arch. Middlesex Hosp., Eighth Cancer Rep.,' 1909, p. 126 and foll.

Reference No.	Substance.	Leak of substance (natural leak = 1).
378	Liver, normal human	1·07
386	„ „	1·01
437*	„ „	0·94
119	Lung „	1·02
125	„ „	1·04
39	Primary sarcoma of adrenal	1·06
87	„ „ femur	0·96
433	Lymphatic glands secondary to carcinoma of rectum	1·14
148	„ „ „ „ breast	0·97
792	„ „ „ „ „	1·09
440*	„ „ „ „ „	1·29
434	Hepatic metastasis „ „ rectum	1·14
576	„ „ „ „ „	0·94
338	„ „ „ „ breast	1·06
790	„ „ „ „ „	1·02
407	Primary carcinoma of bladder	1·04
94	„ „ cervix	1·07
548	„ „ „	0·85
697, Sample A	„ „ „	1·01
697 „ B	„ „ „	1·02
697* „ C	„ „ „	21·28
697 „ D	„ „ „	1·71
697 „ E	„ „ „	1·14
697 „ F	„ „ „	1·10
697* „ G	„ „ „	1·06
71	„ „ ovary	1·02
794	„ „ „	1·06
117	„ „ tongue	1·03
793*	„ „ „	2·24
575	„ „ rectum	1·11
768	„ „ „	1·10
796	„ „ „	0·95
798	„ „ „	0·97
147, Sample A	„ „ breast	1·43
147 „ B	„ „ „	1·34
147* „ C	„ „ „	1·20
442, Sample A	„ „ „	1·67
442 „ B	„ „ „	1·07
789	„ „ „	0·88
791*	„ „ „	1·48
795	„ „ „	1·06
797	„ „ „	0·98

* Tested for emanation.

The results with the emanation electroscope were as below :—

HCl.	Natural leak	0·235 division per minute.
	Experimental leak	0·233 „ „
No. 437.	Natural leak	0·235 „ „
	Experimental leak	0·215 „ „
No. 697 G.	Natural leak	0·192 „ „
	Experimental leak	0·196 „ „

No. 147 C.	Natural leak...	0·192	division per minute.
	Experimental leak ...	0·196	,, ,,
No. 440.	Mean natural leak ...	0·239	,, ,,
	Experimental ,, 1st hour ...	0·410	,, ,,
	,, ,, 2nd ,, ...	0·531	,, ,,
	,, ,, 3rd ,, ...	0·484	,, ,,
No. 791.	Natural leak...	0·221	,, ,,
	Experimental leak, 1st hour...	0·237	,, ,,
	,, ,, 2nd ,, ...	0·267	,, ,,
	,, ,, 3rd ,, ...	0·263	,, ,,
	Natural leak after exhaustion, 1st half hour	0·201	,, ,,
	Ditto, 2nd half hour ...	0·177	,, ,,

(Flask found to be cracked, equilibrium value probably not attained.)

No. 793.	Mean natural leak ...	0·303	division per minute.
	Experimental, "immediately"	1·227	,, ,,
	,, end of 1st hour	1·376	,, ,,
	,, ,, 2nd ,,	1·676	,, ,,
	,, ,, 3rd ,,	1·538	,, ,,
No. 697 C.	Mean natural leak ...	0·318	,, ,,
	Experimental, "immediately"	112·1	divisions per minute.
	,, end of 1st hour	146·3	,, ,,
	,, ,, 2nd ,,	153·8	,, ,,
	,, ,, 3rd ,,	153·8	,, ,,

Standard radium solution containing $1{\cdot}57 \times 10^{-7}$ mgrm. of Ra—

Natural leak ...	0·319	division per minute.
Experimental, "immediately" ...	1·370	divisions per minute.
,, end of 1st hour ...	1·714	,, ,,
,, ,, 2nd ,, ...	2·083	,, ,,
,, ,, 3rd ,, ...	2·143	,, ,,
,, ,, 4th ,, ...	2·000	,, ,,
Natural leak after exhaustion, "immediately"	0·587	division per minute.
Ditto, 1st hour ...	0·412	,, ,,
,, 2nd ,, ...	0·307	,, ,,

Comparing the values obtained by the constant capacity electroscope with the maximum values (*less* mean natural leak) obtained by the emanation electroscope in the case of those substances examined by both methods, it appears that a 20-per-cent. increase over the natural leak in the constant capacity electroscope indicates the presence of radium, but, for values less than this, the presence or absence of radium as detected in the emanation electroscope cannot be predicted with certainty.

Reference No.	Leak of substance (Natural leak = 1). Constant capacity electroscope.	Divisions per minute due to emanation. Emanation electroscope.
437	0·94	0
697 G	1·06	0·004
147 C	1·20	0·004
440	1·29	0·292
*791	1·48	0·068
793	2·24	1·373
697 C	21·28	153·5

* Flask cracked ; equilibrium value probably not attained.

Of the entire series of five non-malignant and 28 malignant tissues that have been examined, it appears, therefore, that three cases of primary carcinoma and one case of secondary carcinoma yield sufficient evidence that in them radium was present. Comparison with the radium standard ($1{\cdot}57 \times 10^{-7}$ mgrm.) shows that they possess the following amounts of radium per gramme of dried extracted carcinoma tissue:—

No. 440.	Spheroidal cell carcinoma, axillary glands secondary to breast	$1{\cdot}08 \times 10^{-8}$ mgrm.
No. 791.	Primary spheroidal cell carcinoma, breast	$0{\cdot}188 \times 10^{-8}$ „ (at least)
No. 793.	„ squamous „ tongue...............	$3{\cdot}94 \times 10^{-7}$ „
No. 697.	„ „ „ cervix	$2{\cdot}73 \times 10^{-5}$ „

A Critical Study of Experimental Fever.

By Edward C. Hort, F.R.C.P. Edin., and W. J. Penfold, M.B., C.M.

(Communicated by Dr. C. J. Martin, F.R.S. Received February 17,—Read March 14, 1912.)

(From the Lister Institute of Preventive Medicine.)

The injection of solutions of salt into man and animals by the subcutaneous or intravenous route has in recent years become a common practice. In many cases the procedure is followed by fever, as was shown in man by Kottmann (1)* and by Schaps (2). If distilled water alone be injected in large quantities the same accident may happen, as shown (3) by E. Bergmann in 1868. In 1910 the injection of small quantities of water into animals was found (4) to have the same effect.

The cause, however, of what are known as "water fever" and "salt fever" has till recently (5) not been satisfactorily explained, though many theories have been advanced.

In 1911 it was suggested by Wechselmann (6) that the fever so often met with in man after injection of sterilised saline containing salvarsan is due to gross bacterial infection of the saline, demonstrable just before sterilisation. This view was based on the discovery of numerous organisms in his unheated solutions, and on the fact that fever no longer followed injection if he dissolved his salt in freshly-distilled water. This suggestion as to the cause of salvarsan fever was also adopted by McIntosh, Fildes, and Dearmont after independent confirmation in Dr. Bulloch's laboratory of Wechselmann's observations. The theory appeared to us to have an important bearing on the wider question of water fever, and of salt fever in general, and we therefore conducted several experiments on animals, publishing (5) our results in December, 1911.

We were able then to confirm the statement that solutions of salt are apt to exhibit pyrogenetic properties that do not belong to solutions made with freshly-distilled water. We found, however, that in the case of water these properties bore an inverse relation to the number of organisms present in the specimens we examined, and by control experiments in animals we showed that to a great extent they were primarily due to the presence in the water of a substance indestructible by prolonged heating at 120° C. We could not remove this fever-producing substance either by filtration through white

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* Numerals in brackets refer to List of References at end of paper.

Doulton filters or by the use of the centrifuge, as shown by subsequent injection of the water. The actual presence therefore of organisms at the time of injection of sterilised saline did not appear to be the sole cause of salvarsan fever, nor of "salt fever" in general.

Since reporting these results we have studied the question more fully. Injection of the centrifugalised deposit from 250 c.c. of water shown to be pyrogenetic, and to contain 73,000 organisms per cubic centimetre, produced no fever. This was also true of a deposit from 75 c.c. of saline shown to be pyrogenetic, and to contain before the use of the centrifuge 950,000 organisms per cubic centimetre. In both cases the medium employed for injection of the organisms was first shown to be free from the heat-stable fever-producing body referred to. On the other hand injection of suitable quantities of water containing this body, but only 40 to 160 organisms per cubic centimetre, produced fever whether salt were subsequently added or not. This body is held back by Martin's* gelatine filter, a fact which shows that it is a colloidal substance in a fine state of dispersion. We conclude, therefore, that contamination by this body of water before mixture with salt is a more important factor in salvarsan fever than we had realised.

The unexpected presence of this hitherto unrecognised body must to some extent vitiate deductions drawn from previous work on the causation of fever which is the sequel to injection of a variety of substances dissolved or suspended in water.

We have therefore determined to what extent the fever which had been known to follow injection of salt, sugar, fibrin ferment, and tissue extracts was due to this contamination.

Experimental Methods.

Before dealing with our evidence, it is necessary to refer to the current belief that it is impossible to study experimental fever with any approach to accuracy. The number of variants is admittedly great. Full experimental data dealing with the most important will be published in due course. They include the effect on body temperature of disease, age, sex, weight, breed, food supply, environment, exercise, rest, repeated thermometric observation, and other factors. Prolonged study has taught us that to establish broad effects, such as the ability of any given substance to produce fever on injection, knowledge of all the variants is necessary but sufficient. On the other hand, no comparative observations can be relied on unless the ratio of the volume of an injection (including weight of material dissolved or suspended in injection fluid) to body weight is kept constant. In every experiment already reported by one of us this injection ratio is stated. In all experiments here cited the same rule is observed. In our experiments we found it necessary to select animals as far as possible of the same age and weight, and to observe constant conditions of food supply and external temperature. All thermometric observations were taken in the rectum every 30 minutes, instruments of tested accuracy being always employed. Every observation has been taken by us, and never entrusted to assistants. The upper normal limit of the daily range of temperature in 150 healthy rabbits was found to be 103·2° F. All animals with a temperature more than slightly above this point were rejected, as well as those showing any apparent departure from health in other ways. Animals less than 1500 grm., or more than 3000 grm., in weight are unsuitable for experiment if the rabbit be used, the former weight indicating too unstable a temperature, the latter too resistant. Reference is here made only to early fever occurring within five hours of injection, and statements as to the

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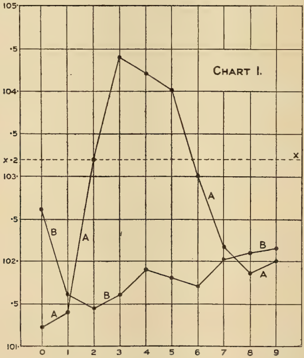
* C. J. Martin, "Gelatine Filter," 'Journ. Physiol.,' 1896, vol. 20.



A. Rabbit, 2448 grm., injected intravenously with 11·50 c.c. water containing F.P.B. Injection ratio, 1/211.

B. Rabbit, 2478 grm., injected intravenously with 11·80 c.c. water containing no F.P.B. Injection ratio, 1/211.

(Interval between observations, 30 minutes.)

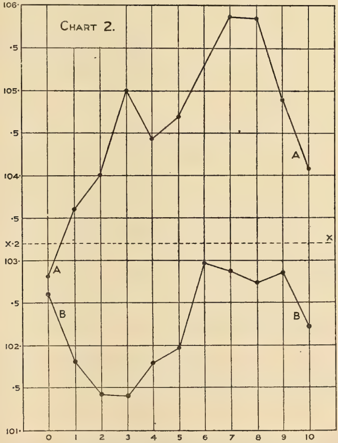
X corresponds to upper normal limit of range of temperature in healthy rabbits. This letter occurs throughout the charts.

presence or absence of fever in any given case are only made in this sense. Unless otherwise stated, all injections were made into the marginal veins of the ear.

The points we wish to establish are illustrated by charts. For the sake of brevity, we designate the fever-producing body under discussion by the letters F.P.B.

Water Fever.

Charts 1, 2, 3 are typical instances of experiments showing the fever-producing capacity of ordinary distilled water, and its absence in the case of water freshly distilled from a glass retort and injected at once. The essential features of these charts occurred in every one of several hundred experiments in which different volumes per kilogramme were injected. Special stress is laid not only on the absence of fever in the control animals, but also on the

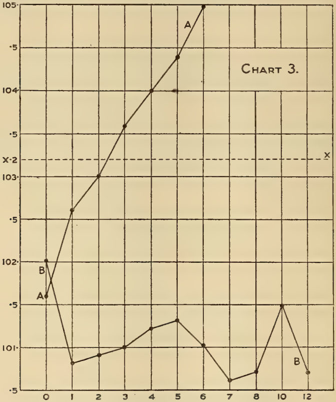


A. Rabbit, 2926 grm., injected intravenously with 50·40 c.c. water containing F.P.B. Injection ratio, 1/58.

B. Rabbit, 2500 grm., injected intravenously with 50 c.c. water containing no F.P.B.

(Interval between observations, 30 minutes.)

marked fall of temperature. The different extent of fever excited by different injection ratios when one sample of water containing F.P.B. is used, is clearly seen in nearly all the charts here shown. In 1910 one of us (4) advanced the theory that water fever is largely an auto-intoxication due to absorption of lytic products liberated locally by the injection of water. The evidence given in support of this view now disappears, since water supposed to be pure, and known to contain only 160 organisms per cubic centimetre, may actually contain F.P.B.



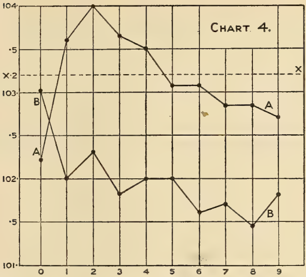
A. Rabbit, 2083 grm., injected subcutaneously with water containing F.P.B. Injection ratio, 1/40.

B. Rabbit, 2080 grm., injected subcutaneously with water containing no F.P.B. Injection ratio, 1/40.

(Interval between observations, 30 minutes.)

Salt Fever.

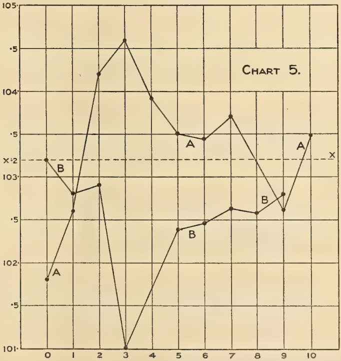
Charts 4 and 5 show that solutions of salt made with ordinary distilled water give rise on injection to fever, but that when freshly distilled water is the solvent a fall of temperature results. These effects were obtained in all our experiments up to 25-per-cent. concentrations of sodium chloride.



A. Rabbit, 1970 grm., injected intravenously with saline made with water containing F.P.B. Injection ratio, 1/211.

B. Rabbit, 1800 grm., injected intravenously with saline made with water containing no F.P.B. Injection ratio, 1/211.

(Interval between observations, 30 minutes.)



A. Rabbit, 2551 grm., injected intravenously with saline made with water containing F.P.B. Injection ratio, 1/350.

B. Rabbit, 2316 grm., injected intravenously with saline made with water containing no F.P.B. Injection ratio, 1/211.

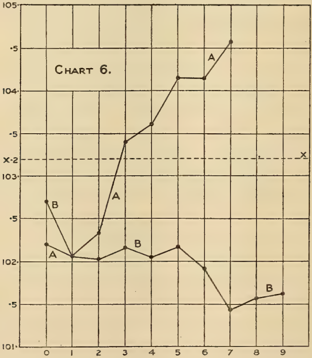
(Interval between observations, 30 minutes.)

In the experiments illustrated by the curves A the water injected was ordinary distilled water and contained 40 organisms per c.c. In the case of the experiments illustrated by curves B, the saline was made with freshly distilled water and at once injected. The results here recorded occurred in all essentials in all of the 27 experiments made. Numerous papers dealing with salt fever have appeared (7–14) in which various theories based on the view that salt was the pyrogenetic agent have been advanced. The evidence in support of these views no longer holds good.

Carbohydrate Fever (Charts 6, 7, 8).

Charts 6 and 7 show that the injection of glucose and saccharose produced no fever when the solutions injected were made in pure water.

Chart 8 shows temperatures obtained in two experiments made with lactose from different sources. The solutions were made in pure water. The upper curve shows the result with a sample of commercial lactose. The lower curve shows the result with lactose made from a catheter sample of cow's

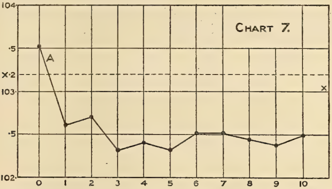


A. Rabbit, 1545 grm., injected intravenously with 8 c.c. 5-per-cent. glucose in water containing F.P.B. Injection ratio, 1/193.

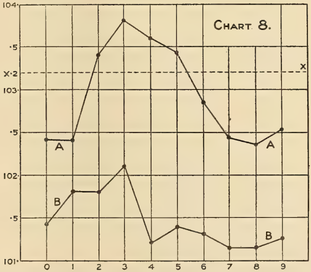
B. Rabbit, 1718 grm., injected intravenously with 8—9 c.c. 5-per-cent. glucose in freshly distilled water. Injection ratio, 1/193.

(Interval between observations, 30 minutes.)

milk. In its preparation all the reagents used were made with freshly distilled water, and the crystallisation was carried out at 0° C. For this we are indebted to Dr. McLean at the Lister Institute. With these precautions the rise of temperature was insignificant.



A. Rabbit, 1747 grm., injected intravenously with 8·8 per cent. cane sugar dissolved in 8·20 c.c. water freshly distilled from glass retort. Injection ratio, 1/211.
(Interval between observations, 30 minutes.)



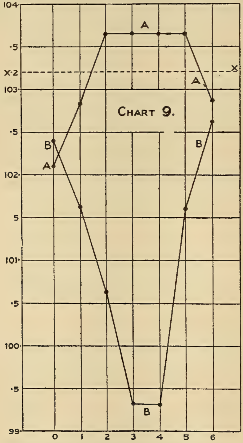
A. Rabbit, 1647 grm., injected intravenously with isosmotic solution of commercial lactose in pure water. Injection ratio, 1/211.

B. Rabbit, 1605 grm., injected intravenously with isosmotic solution of lactose obtained from a catheter sample of milk in the method described in text.
(Interval between observations, 30 minutes.)

The existence of carbohydrate fever (10, 15, 16), which has long been looked upon as a definite clinical type, is not supported by the above experiments.

Tissue Fever (Charts 9, 10, 11).

Chart 9 shows that the presence of F.P.B. is also responsible for the fever that follows the injection of blood laked with water, and that injection of similar quantities of blood laked with pure water produces marked fall of temperature. This fall, as will be seen, is due to the depressant effect of two distinct factors, pure water and extract of red cells.

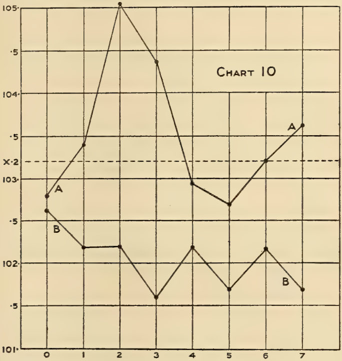


A. Rabbit, 2590 grm., injected intravenously with 4·6 c.c. rabbit blood in 15·3 c.c. water containing F.P.B. Injection ratios, 1/563 and 1/169.

B. Rabbit, 2197 grm., injected intravenously with rabbit blood in pure water. Injection ratios as in A (slightly less).

(Interval between observations, 30 minutes.)

Chart 10 shows the relative temperatures after injection of two animals with extract of red blood cells taken from another animal of the same species. The extract was made in the case of animal A by lysis of the cells in water shown to contain F.P.B., the extract for animal B being made in pure water. This chart destroys the value of the evidence of fever due to injection of extract of red cells of the same species. The same result followed the injection of red-cell extract of another species, as well as that of red-cell extract made from the cells of the animal injected. In all cases where fever resulted the water used for the preparation of the extracts was the same and contained 40 organisms per c.c. on injection. The water employed in the preparation of the sodium citrate solutions into which the blood was shed, the saline in which the cells were washed, and for lysis of the blood corpuscles was a sample containing F.P.B., and 40 organisms per c.c. The control extracts were made throughout with solutions containing pure water. The extent of fever shown is in excess of that which follows injection of a similar quantity of water containing F.P.B. This increase is perhaps due to absorption of F.P.B. during preparation of the extracts, perhaps to the action of F.P.B. on the stromata resulting in their acquisition of pyrogenetic function not previously possessed. The point requires quantitative investigation.

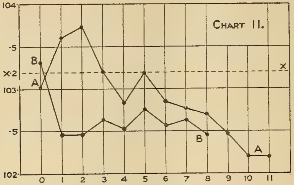


A. Rabbit, 2785 grm., injected intravenously with red-cell extract in water containing F.P.B. Injection ratios of water and extract same as in B (figures lost).

B. Rabbit, 2370 grm., injected intravenously with red-cell extract in water containing no F.P.B. Injection ratios of water and extract same as in A.

(For details of preparation of extract, *vide* text. Intervals between observations, 30 minutes.)

Chart 11 shows that the injection of fresh normal serum produces a fall of temperature, in spite of the presence of fibrin ferment. This fact gravely affects the theory of fibrin ferment fever (17–22) more especially as all the evidence hitherto accepted in support of this theory is based on injection of extracts in water that we now know to have been liable to contamination with F.P.B. Until, therefore, these experiments on ferment fever, including fibrin ferment fever, have been repeated with extracts in water proved to be free from this substance all observations on this subject require careful scrutiny.



A. Rabbit, 2211 grm., injected intravenously with 3 c.c. normal serum from another healthy rabbit. Injection 4 hours after separation. Injection ratio, 1/737.

B. Rabbit, 1932 grm., injected intravenously with 3·50 c.c. of his own serum. Injection 4 hours after separation. Injection ratio, 1/552.

(Interval between observations, 30 minutes.)

With regard to Charts 9, 10, 11 as a group it is generally believed that the injection of sterile extracts of animal tissues (23–25) may cause fever. This applies to extracts of foreign blood, of red cells, of leucocytes, or of blood serum, as well as to extracts of all foreign tissues. By the term foreign tissue is here meant tissue alien to the animal injected, even if belonging to animals of the same species. Fever is also believed to follow the injection of an animal with extracts of his own blood or blood cells, and the fever following fracture and hæmatomata have been thus explained. In all experiments, however, the extracts were prepared in liquids containing water which must now be regarded as suspect. In view of our experiments the value of much of the evidence, therefore, on which belief in this type of fever rests disappears. If reference be made to Charts 1, 2, 3 in the light of Charts 9, 10, 11 it will be seen that they afford additional evidence that the theory of tissue fever cannot be supported by lysis of cells effected by the injection of distilled water. We find this to be also true of extracts of solid organs, including the brain. The injection of fresh egg albumen also failed in our hands to give rise to fever, a fact that has some bearing on the belief in protein fever after single injections of this class of substance in an unbroken state.

In conclusion, we submit that the existence of "water-fever," "salt-fever," "sugar-fever," "ferment-fever," and "tissue-fever" no longer rests on secure ground. That future advance in the study of fever is only possible by recognition of all the fallacies inherent to experiments involving the use of water liable to contamination with this fever-producing substance will not, we believe, be questioned.

The fact that one of us has been a victim to these fallacies is sufficient justification for the critical nature of this note.

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On the Systematic Position of the Spirochæts.

By Clifford Dobell, Fellow of Trinity College, Cambridge; Lecturer at the Imperial College of Science, London, S.W.

(Communicated by A. Sedgwick, F.R.S. Received February 21,—Read March 28, 1912.)

This paper is a very brief summary of the chief results of my researches on the Spirochæts and related organisms. I have been occupied with these researches for several years, and I believe that I have now obtained sufficient evidence for it to be possible to form a correct judgment regarding the systematic position of the Spirochæts.

It is well known that various views of this problem have been taken. Hitherto, three different opinions have been expressed,* and more or less vigorously defended, by different workers. They are: (1) that the Spirochæts belong to the Protozoa; (2) that they belong to the Bacteria; (3) that they belong to the Cyanophyceæ. The upholders of the first view suppose that the Spirochæts resemble the Flagellata, especially the trypanosomes. Those who support the second view usually regard the Spirochæts as being closely similar to *Spirilla*. Those who support the third view believe that the Spirochæts show points of resemblance to the spiral forms of Cyanophyceæ (*Spirulina* and *Arthrospira*).†

In view of the existence of these wide differences of opinion and the corresponding mental attitudes of those who have attempted to form a judgment in this matter, I would submit the following statement regarding my own position:—

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* There is a fourth view, which I formerly advocated—namely, that the Spirochæts should be regarded as an independent group of organisms. I no longer hold this view, as further research has shown me that it is incorrect.

† These three views may be traced back respectively (1) to Schaudinn (1904); (2) to Ehrenberg (1833), who placed *Spirochæta* in his family Vibrionia—the equivalent of the modern Bacteria; (3) to Cohn (1854), who regarded *Spirochæta* as a colourless form of *Spirulina*.

First, concerning the criteria which may be employed to decide the systematic position of the Spirochæts. There is, in my opinion, but one kind of evidence which can lead to a correct verdict. Judgment—if it is to be conclusive—must be given upon morphological evidence. The classification of all living beings is based ultimately upon morphology—using the word in its widest sense to mean the study of the forms of organisms at all stages in their life-histories. If the Spirochæts are to enter our artificial system of classification, they must do so on the same terms as other organisms. "Systematic position" must be judged by the same standard throughout, otherwise the system would be inconsistent, and the term would cease to have any definite meaning. It is, of course, possible to characterize the Spirochæts by certain of their physiological, pathological, or chemical attributes. But to attempt to enrol them in the system of organized beings by virtue of such attributes alone would involve the reclassification of all other living beings in the same terms.

Secondly, I think the following postulate must also be granted. To determine the systematic position of any group of organisms it is necessary to have accurate knowledge of their structure and life-histories; and we must have this knowledge not only of the group in question, but also of all the other groups which it may conceivably resemble. This means, in the present case, that to determine the systematic position of the Spirochæts it is necessary to possess accurate knowledge of the morphology of the Spirochæts, the Flagellata, the Bacteria, and the Cyanophyceæ. Without such knowledge it is not possible to assign the Spirochæts a definite place in the present system of classification.

These two postulates are, to my mind, so indisputable that they are almost platitudes. Nevertheless, it has become apparent to me, from the work of others, that they are neither recognised nor accepted universally. I have particularly emphasized them because my conclusions as to the systematic position of the Spirochæts depend upon the recognition of their validity.

Desiring to determine the systematic position of the Spirochæts, I have, in accordance with my convictions, endeavoured to study the morphology of all the chief types of Spirochæt. Four different genera of these may be distinguished*—*Spirochæta* Ehrenberg, *Cristispira* Gross, *Treponema* Schaudinn, and *Saprospira* Gross. The four genera may be collected into a single group which I have called the Spirochætoidea. I have made a careful study of the morphology of two species of *Spirochæta* (from fresh water); four species of *Cristispira* (from Lamellibranchs); nine species of *Treponema* (eight parasitic forms from various animals and one free-living form from fresh water); one species of *Saprospira* (from fresh water). In addition to these, I have studied in a similar way a large number of flagellate Protozoa, Bacteria, and Cyanophyceæ. I have devoted special attention to the forms which are of special importance in the present connection. These are certain spirillar and bacillar forms of bacteria, and the spiral forms (*Spirulina*, *Arthrospira*) of Cyanophyceæ. As nearly all these organisms are extremely small, I have naturally paid considerable attention to matters of cytological and microscopic technique. I shall not describe here the methods which I have used, as my aim now is to give my results only.

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* Gross is in complete agreement with me regarding the existence of these four different types. But he regards *Spironema* as the correct name of the set of forms which I call *Treponema*. In addition to these two names, more than half-a-dozen others have been given to these organisms—collectively or severally. I may mention the following:—*Spirochæta*, *Spirillum*, *Vibrio*, *Trypanosoma*, *Microspironema*, *Borrelia* *Spiroschaudinnia*. The nomenclature is still in great confusion.

The morphological characters which I have been able to establish in the case of the Spirochæts which I have studied are the following:—

(1) All the Spirochæts possess an elongate and more or less spirally wound body, which is non-cellular.*

(2) They are flexible—the degree of flexibility displayed differing in different species.

(3) Antero-posterior polarity is absent in all genera. In other words, during movement either end of the body may function as an anterior or posterior end.

(4) The body is clothed with a pellicle, the thickness of which differs in the different genera. (In the smallest species of *Treponema* and *Spirochæta*, the presence of a pellicle cannot be established with certainty.)

(5) All the Spirochæts which are sufficiently large for accurate observations to be made in this respect are plasmolysable. (The results of plasmolysis are not identical in all forms. The differences observed appear to be due to corresponding differences in the thickness and nature of the pellicle.)

(6) No flagella or other special organs of locomotion are present.

(7) The cytoplasm possesses a peculiar structure—which I have called† the "chambered structure"—and there is probably a form of chromidial nucleus present. This is true of *Cristispira*, *Saprospira*, and the largest form

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* I cannot agree with Gross, who maintains that the Spirochæts are *multicellular*. I believe they are all non-cellular ("unicellular").

† I have described this in detail in a previous account of *Cristispira veneris* ('Quart. Journ. Micro. Sci.,' 1911, vol. 56). My description is essentially similar to those previously given (of other species of *Cristispira*) by Laveran and Mesnil, Swellengrebel, Schellack, and Gross—the differences being in matters of detail.

of *Treponema* which I have studied. (A chambered structure cannot be demonstrated with certainty in the small species of *Treponema* and *Spirochæta.*)*

(8) In *Cristispira* there is a peculiar axial thickening of the pellicle—the crista. I regard this as the homologue of the axial fibre of *Spirochæta plicatilis.* No similar structure exists in *Saprospira* and the largest forms of *Treponema.* (In the smallest species of *Spirochæta* and *Treponema,* it is not possible to determine whether any structure of this sort is present or not.)

(9) Metachromatic granules ("volutin" granules) are present in *Cristispira, Saprospira,* and *Spirochæta.*† Possibly they occur in *Treponema* also, but I have not succeeded in demonstrating their presence or absence in these forms on account of their very small size.

(10) Colouring matter (chromophyll) is absent.‡

(11) Cyanophycin granules are absent.§

(12) The method of multiplication is always by transverse division—division being simple in *Cristispira* and *Treponema,* multiple in *Saprospira* and *Spirochæta.*

(13) Correlated with this method of multiplication there is a marked peculiarity in the variation of the dimensions of the body, in all four genera of the Spirochæts. The individuals of any species show a considerable variability in length—due to growth in one dimension of space—but not in breadth.

I may add that I have obtained no evidence of spore-formation or sexual processes in any Spirochæt, and I regard as inconclusive all the evidence adduced by others to prove the existence of these phenomena.

If the Spirochæts be compared, in respect of the characters enumerated above, with the Flagellata, it will be found that the only common characters are those numbered 2, 4, 9, 10, 11. But it will also be found that these characters are common to a very large number of Protista. The differences, on the other hand, are—in my opinion—so pronounced as to exclude the Spirochæts not merely from the Flagellata, but from the whole group of the Protozoa. The combination of characters numbered 1, 3, 5, 7, 8, 13 does not occur in any known protozoon.

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* From Zülzer's just published account of the large *Spirochæta plicatilis*—a form which I have not studied in detail—it appears that this organism also possesses protoplasm with a chambered structure.

† They have been demonstrated in *Spirochæta plicatilis* by Zülzer. I have not succeeded in demonstrating them in the two much smaller species which I have studied.

‡ This is present in all Cyanophyceæ.

§ They are present in all Cyanophyceæ except *Spirulina*—so far as I have been able to determine.

I have studied a large number of forms belonging to the Cyanophyceæ.* Those which are of special interest in the present case are the spiral forms —*Spirulina* and *Arthrospira.* I have made a detailed study of a marine species of the former (*S. versicolor* Cohn), but have been able to make only fragmentary observations on *Arthrospira.* I have found that both organisms differ so markedly from all the Spirochæts that I regard the possibility of any close relations between them as excluded. The differences concern especially the cytoplasmic and nuclear structures, and the method of multiplication (in addition to the movements and habits in general). The similarities observable in the Spirochæts and the spiral forms of Cyanophyceæ are merely in respect of certain features common to nearly all the Schizophyta (Bacteria and Cyanophyceæ). In agreement with Gomont, I regard *Arthrospira* as a member of the Oscillatoriaceæ: whereas *Spirulina* occupies an isolated position among the other Cyanophyceæ. I have not encountered the remarkable organisms named *Glaucospira* by Lagerheim, and I am unable to draw any certain conclusions concerning their systematic position from the very unsatisfactory accounts of them which have been published.

From the standpoint of morphology, therefore, I believe there is no justification for the views that the Spirochæts belong to the Flagellata (or even to the Protozoa), or that they belong to the Cyanophyceæ. On the other hand, I think the evidence is conclusive that they cannot be placed in either of these groups.

If, however, the Spirochæts be compared with the Bacteria,† in respect of the characters enumerated above, it will be found that very striking similarities exist between the two groups. The characters numbered 1, 3, 4, 5, 9, 10, 11, 12, 13 are all more or less widely recognised characters of certain of the Bacteria, many of them being characteristic of the group as a whole. Concerning the remaining Spirochæt characters I may add:—

(*a*) With regard to (2), I have been able to show that many parasitic and free-living bacillar forms of bacteria and at least one spirillar form (*Paraspirillum*) are flexible.

(*b*) With regard to (7), I have been able to show that a chambered structure of the protoplasm occurs in certain spirillar and bacillar forms of bacteria, both parasitic and free living.

(*c*) With regard to (8), I have found in certain *Spirilla* that there is an axial thickening of the pellicle which I believe to be homologous with the crista of *Cristispira* and the axial fibre of *Spirochæta.*

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* My researches on the cytology of the Cyanophyceæ have not yet been published, as they are not yet finished.

† My earlier studies on the Bacteria have already been partly published. See especially my "Contributions to the Cytology of the Bacteria," 'Quart. Journ. Micro. Sci.,' 1911, vol. 56.

In fine, I believe that I have been able to show that, with one exception, all the chief characters of the Spirochæts are to be found represented in the Bacteria. Beyond a doubt, the Spirochætoidea resemble both the bacillar and and spirillar forms of bacteria very closely. There is but one peculiar character which the Spirochæts possess—namely, the power of active movement without the aid of flagella or other organs of locomotion. No known bacteria, so far as I am aware, possess such a power.

As I believe I have been able to establish all the foregoing points in the morphology of the Spirochæts and the Bacteria, I think I am justified in drawing the conclusion that the two groups of organisms cannot be separated systematically. In other words, the Spirochæts must be classified with the Bacteria.

The group of the Haplobacteria, as at present constituted, contains three different sets of forms—bacillar, spirillar, and coccus forms of bacteria. The distinction between these is, in all probability, in many cases purely arbitrary, owing to the fact that many of the Bacteria are pleomorphic. For systematic purposes, however, it is convenient to subdivide the Haplobacteria into these three groups, which I may call Bacilloidea, Spirilloidea, and Coccoidea. I believe that the Spirochætoidea should, for the present, be regarded as a group of the same systematic status—whatever that may be. I fully realise that the classification of the Bacteria is, at present, impossible. It will be possible only when the morphology and life-histories of these organisms are more fully known.

It is possible that many Spirochæts are transient forms of pleomorphic organisms. I would suggest that the "granules" and "spores" described by several workers in several different Spirochæts are really coccus forms of these bacteria.

The systematic position which I would assign to the Spirochæts is shown in the following scheme:—

- SCHIZOPHYTA
  - Cyanophyceæ
  - BACTERIA
    - Trichobacteria
    - HAPLOBACTERIA
      - Coccoidea
      - Bacilloidea
      - Spirilloidea
      - SPIROCHÆTOIDEA
        - *Spirochæta.*
        - *Treponema.*
        - *Cristispira.*
        - *Saprospira.*

A full account of my researches—with illustrations, a detailed analysis, and references to the literature—will appear later.

The Production of Variation in the Physiological Activity of Bacillus coli *by the Use of Malachite-Green.*

By Cecil Revis.

(Communicated by Sir J. R. Bradford, Sec. R.S. Received February 22,—Read March 28, 1912.)

In a paper published last year (1) I showed that by the cultivation of a typical *Bacillus coli* in peptone broth containing steadily increasing quantities of malachite-green, the organism could be trained to develop without difficulty, even in the presence of 0·1 per cent. of the dye, but that, at the same time, the organism underwent a profound change, and lost the power of producing gas in certain sugars and polyhydric alcohols, the attack ceasing at the acid stage. The properties of the organism so produced were of a permanent character.

As the change was at the moment unique, it was necessary to produce, if possible, the same change in other organisms of the same type, on account of the far-reaching deductions which may be made from this variation. In the case recorded, however, the possibility of contamination was practically excluded by the precautions employed, while mathematically it was almost negatived.

Four organisms (three being typical strains of *Bacillus coli*, and the fourth *Bacillus acidi lactis* Hueppe) were carefully plated out, and grown for periods of seven days in peptone broth (reaction + 1) to which increasing amounts of malachite-green had been added until 0·10 per cent. was present. The cultures were incubated at 37° C. After a suitable interval (about 15 to 20 reinoculations carried out with every precaution against accidental contamination) the cultures were plated out and examined for physiological activity.

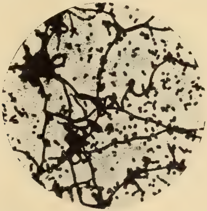
Of the four organisms, which may be distinguished as *Coli* A, B, and C, and *Hueppe*, the two latter showed practically no change, though some of the reactions were delayed. Considerable variations in the type of colony were observed, but this is quite usual. The four organisms showed very varying resistance to the malachite-green, *Hueppe* and *Coli* B were very susceptible, and had to be carefully acclimatised, while the other two found no difficulty in development in presence of the dye. The susceptibility appears, however, to have no bearing on the physiological change.

The other two had, however, undergone exactly the same change as that previously recorded, and the various colonies examined consisted of organisms which produced acid in the same test media as the original cultures (which, it may be remarked, had undergone no change when tested again at the conclusion of the experiment), but the power to produce gas had been completely lost, and could not be restored by repeated sub-culture in sugar media.

Coli B.—This organism had been comparatively recently isolated from horse-dung, and was quite typical in every way. Beyond the change in physiological activity, it showed scarcely any difference from the original culture, except that, on microscopic examination, the presence of long filaments was noticed, greatly in excess, both quantitatively and qualitatively, of that usually associated with *Bacillus coli.*

Coli A.—This organism was particularly included, because, though quite typical, it had been kept in the laboratory for nearly four years, and was showing a very slight but noticeable change in physiological activity.

Besides the physiological change already mentioned, it showed profound morphological and cultural variation. Colonies grew slowly (not appearing before the fourth day) on gelatin or agar at 20°, and then appeared as large, viscous, circular masses, consisting of a mixture of very long filaments and short bacilli, together with a gummy cementing substance (see figure). By no possible means could the presence of a mixture be detected, and there is no doubt that the filaments and bacilli belong to the same culture.



The action of the malachite-green, therefore, has been to produce an organism from a typical *Bacillus coli*, which is *neither physiologically, morphologically, nor culturally a colon bacillus.*

The identity of the organism throughout the experiment is easily seen from the character of the change produced, and the concomitant filamentous growth and slime production are not without precedent. I have shown elsewhere that *Bacillus coli*, when grown under certain conditions such as in soil, mineral media, etc., develops the power of slime production in an extraordinary manner (2).

Bacillus coli at all times shows a tendency to grow into filaments, though not of very great length. In the course of some experiments, now proceeding, in which methylene-blue is substituted for malachite-green, the development of long filaments of similar character to those produced in *Coli* A has been noticed in several cases.

This peculiar type of growth, together with the production of "gum," seems to point strongly to the development of *Bacillus coli* from a class of well-known soil bacteria, and, in fact, I have been able to train some of these bacteria to a very close resemblance to the new type developed from *Coli* A under the action of malachite-green.

I am indebted to the kindness of Dr. Vincent, Senior Physician to and Director of the Bacteriological Laboratory of the Infants' Hospital, Vincent Square, for the photograph of *Coli* A here reproduced.

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The Influence of Selection and Assortative Mating on the Ancestral and Fraternal Correlations of a Mendelian Population.

By E. C. Snow, M.A., D.Sc., Biometric Laboratory, University College, London.

(Communicated by Prof. Karl Pearson, F.R.S. Received March 1,—Read March 28, 1912.)

The actual effects of selection and assortative mating on the variability and correlation of organs have long been known. The general numerical resemblance between the correlations found for gametic characters on the Mendelian hypothesis for random sampling and mating and those computed from actual data is now also well established, though the fundamental difficulties in the way of reconciling the deductions from that hypothesis with the distributions and regressions actually obtained for, say, coat-colour in mice, have not been diminished. It is of importance, therefore, to ascertain what conclusions can be reached as regards the numerical effects of selection and of assortative mating on the basis of the Mendelian mechanism, and to this end an analytical investigation was entered upon on a more general and rigorous foundation than any which have previously been used to attempt to solve the problem.

Dealing first with the correlations for gametic characters. In general terms, it was established that the effect of taking a selected sample instead of a random one from a population showing a zero coefficient of assortative mating would be to find the ancestral and fraternal correlations within that sample less than those of a random sample, so long as the variability was diminished by the selection. If an ancestor be selected, the correlations between that ancestor and descendants diminish in geometrical progression. On the whole, selection of parents appears to affect the correlations between them and their offspring to a greater extent than it affects the relationship between those offspring themselves. For all ancestral cases the regressions appear to be more stable properties of a particular population than the corresponding correlations; frequently the regression of offspring on ancestor is the same as for a random sample, though the correlation is changed. The investigation also brought out that the value 0·5 in each case for the fraternal and parental correlations obtained when random samples of a general Mendelian population are dealt with does not depend upon the fact that the samples are random ones, but upon the fact that for such a population the frequency of the heterozygote is twice the geometric mean of the frequencies of the dominant and recedent homozygotes. For, if any *selected* sample of the form $p_1^2(\mathrm{AA}) + 2p_1q_1(\mathrm{A}a) + q_1^2(aa)$ be taken from the general population $p^2(\mathrm{AA}) + 2pq(\mathrm{A}a) + q^2(aa)$, the parental and fraternal correlations reached when the individuals of the selected sample mate at random within the sample always have the constant value 0·5.

The investigation next considered the question of assortative mating within a random sample of the general population. Such mating, if positive, increases the parental and sibling regressions as well as the correlations. The ancestral regressions diminish in geometrical progression, the correlations not perfectly, but nearly so. In certain cases the expressions found for the parental and sibling correlations were identical with those reached by the very general methods previously employed by Pearson, and which have no connection whatever with Mendelism, but this can hardly be more than a curious coincidence.

When we deal with assortative mating within a selected sample, we find the regression of offspring on parent depends upon both the assortative mating and the intensity of selection, and increases as those factors increase. Selection and assortative mating affect the correlations in opposite directions, the decreasing tendency of the former appearing to have the predominating effect in practical cases. The sibling correlation is not raised so much by assortative mating nor reduced so much by selection as is the parental one.

Fairly similar qualitative results were found throughout for somatic characters, though not so much weight can be given to them as to those for gametic characters. Moreover, it is the latter which agree most closely with observation. It is to the results for gametic characters, therefore, that we must look for theoretical verification for experimental conclusions which, at first sight, appear paradoxical, *e.g.* the closeness of the resemblance between cousins.

The Nature of the Immune Reaction to Transplanted Cancer in the Rat.

By Wm. H. Woglom, M.D.

(Communicated by Sir J. R. Bradford, Sec. R.S. Received March 15,—Read May 2, 1912.)

The purpose of the present paper is to extend to another species the investigation of the early history of tumour grafts carried out by Russell* in normal and immune mice, and to describe in detail the reactions taking place in and about fragments of the Flexner-Jobling† adeno-carcinoma of the rat after its transplantation into normal and resistant animals.

Immune rats were obtained by selecting those which had proved themselves resistant to one or two inoculations of the tumour in question, or by subjecting animals to previous treatment with 0·2—0·3 c.c. of an emulsion of rat embryo skin, and the introduction of the grafts destined to be excised for the study of early stages took place in all cases two or three weeks after the last immunising treatment.

Fragments removed after 24 hours show commencing degeneration of the stroma and of the parenchymal elements toward the central parts, while the tumour cells at the periphery have, on the contrary, preserved their vitality. There is no visible difference at this time between grafts removed from immune rats and those from normal animals.

The most interesting epoch in the history of a tumour nodule which is establishing itself in a new soil is the third day. In the majority of fragments removed from normal rats at this period the fibrinous exudate and the cleft, both formerly separating the graft from its host, have disappeared. The fibroblasts of the surrounding connective tissue have entered into the growth, and are engaged in the building of a new stroma, while penetrating capillaries can be discovered at the edge of the young tumour. The outermost cells of the parenchyma are in active mitosis, but the centre of the graft is quite necrotic. In fig. 1, representing the edge of a nodule which was removed 70 hours after implantation, the close connection between the growth and its host can be readily appreciated from the intimate commingling of tumour cells and connective tissue elements, as they are reproduced in the drawing. A new capillary, containing blood, has entered well into the fragment, and is indicated toward the lower edge of the illustration to the right of the centre. The hyaline remains of the old stroma, its penetration by polymorphonuclear leucocytes, and the serious involvement of its connective tissue cells are also reproduced, as well as the active mitosis that is in progress among the elements of the parenchyma at the growing edge of the graft; but the necrotic tumour cells in the centre of the fragment are not included in the picture. The surviving cells of the parenchyma, which have hitherto been content merely to sustain life and to proliferate as best they may, now often show signs of an attempt to assume an acinous arrangement.

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* Russell, B. R. G., 'Third Scientific Report, Imperial Cancer Research Fund, London,' 1908, p. 341.

† Flexner and Jobling, 'Monographs on Medical and Allied Subjects, Rockefeller Institute,' New York, 1910, No. 1, p. 1.

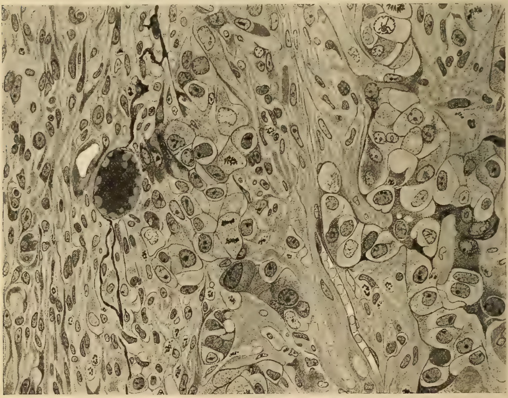

Fig. 1.—Flexner-Jobling adeno-carcinoma. Graft in normal rat 70 hours after implantation. Entrance of new blood vessels and fibroblasts from the host (on left). Mitoses in parenchymal cells, old stroma degenerated. Borrel, iron alum hematoxylin. × 410.

Whether the new framework is derived entirely from the connective tissue of the host, or whether certain cells of the transplanted stroma survive long enough to participate in its construction, is a question difficult of decision. Certain it is that all of the elements of the transferred stroma seem to be considerably damaged before the entrance of connective tissue corpuscles from the host; still the possibility cannot be eliminated that the damaged cells may in some cases be able to recover and continue their proliferation for a time. Beyond reasonable doubt, however, nearly all of the introduced stroma perishes within the first few days, so that the framework of the new tumour is entirely, or almost entirely, the product of the host. This view coincides with that of Flexner and Jobling, who have expressed the opinion that only the epithelial cells of this growth survive transplantation, that the new tumour is the result of their proliferation, and that its stroma is furnished by the connective tissues of the host.

There is little need to insist upon the contrast between the condition just described and that represented in fig. 2, which reproduces a graft taken from an immune rat 72 hours after inoculation. The outlying cells of the parenchyma are still well preserved at this period, and division figures are not infrequent, but there is no trace of acinous arrangement, and the cells lie either as irregular groups within the lacunæ of the stroma, or else in single layers at the edges of the cleft. The imbedded fragment is shrunken, it remains entirely separated by a space and a barrier of fibrin from the

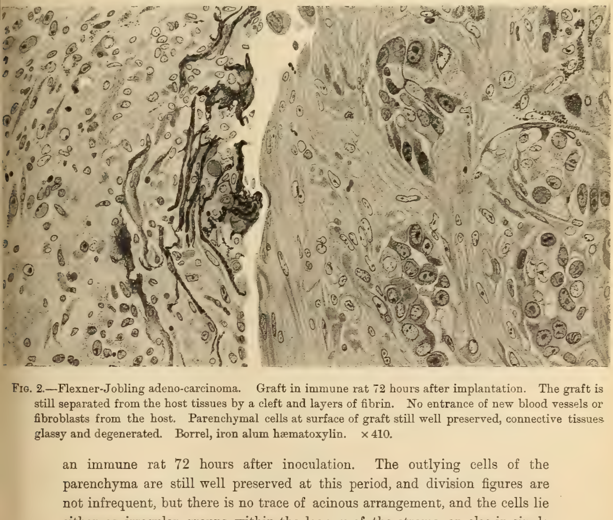

Fig. 2.—Flexner-Jobling adeno-carcinoma. Graft in immune rat 72 hours after implantation. The graft is still separated from the host tissues by a cleft and layers of fibrin. No entrance of new blood vessels or fibroblasts from the host. Parenchymal cells at surface of graft still well preserved, connective tissues glassy and degenerated. Borrel, iron alum hæmatoxylin. × 410.

neighbouring connective tissues of the host, and nowhere can there be detected that projection of fibroblasts between the cells of the tumour which invariably occurs in successful grafts. In many cases the fibrin barrier is even more persistent than in the specimen from which the drawing was made.

After the third day, the graft in a normal animal is the theatre of a progressive and orderly vascularisation and formation of stroma. By the fifth, an acinous arrangement of the parenchymal cells is practically completed, and at the seventh day, the production of collagen having commenced in the new stroma, the implanted tumour is well on its way toward maturity.

Widely different conditions, on the contrary, obtain in fragments which have been introduced into resistant rats. After the third day, degeneration of the graft becomes progressively more serious, and, in the absence of the specific stroma reaction, necrosis is complete by the tenth, although those cells more fortunately situated at the margin of the fragment are able to survive, and in a few cases even to proliferate, as long as eight days after transplantation.

The investigation leads inevitably to the conclusion that the phenomena described by Russell as characterising the immunity of mice to tumour implantation occur also in the case of the resistance offered by rats. Furthermore, as no difference could be detected between grafts taken from rats treated with embryo skin, and those removed from animals which had undergone a previous unsuccessful inoculation with tumour, it is concluded that resistance is similar in the two cases. Both types are the outcome of a failure on the part of the new host to furnish to the implanted fragment the proper blood vessel supply and connective tissue scaffolding.

The expenses of this investigation were borne in part by the Crocker Cancer Research Fund of the College of Physicians and Surgeons, Columbia University, New York City.

The Manifestation of Active Resistance to the Growth of Implanted Cancer.

By B. R. G. Russell, M.D.

(Communicated by Sir John Rose Bradford, Sec. R.S. Received March 15,—Read May 2, 1912.)

(From the Laboratory of the Imperial Cancer Research Fund.)

The current classification of neoplasms has been almost entirely founded upon their structural characters, qualified wherever possible by the knowledge available upon their histogenesis. The researches which have been made, more especially with transplanted tumours of the rodents, have superadded to the recognised morphological differences and resemblances which tumours exhibit, much information upon the more physiological side of their activities, and have shown how variable tumour strains may be in their manner of growth. In the present communication, the individuality of various transplantable tumour strains will be brought out by subjecting them to an analysis with a new factor. This factor will be the quality of the reaction which a strain induces when implanted into a fresh series of animals, and this reaction will be tested by showing how the animals behave to subsequent inoculation of a transplantable tumour. In essence, therefore, the test applied is one for the presence or absence of a soil suitable for the sowing of tumour cells. The completion of this analysis will be followed by the exposition of a series of experiments bearing more especially upon the range of action of the unsuitability of the soil which can be artificially induced.

The propagation during an extended period of over 80 different tumour strains in the laboratories of the Imperial Cancer Research Fund* has provided an extensive material, presenting very different types of growth, and suitable for a comparative analysis. Exactly what is conveyed by the term, type of growth, will be apparent from the two accompanying figures of tumour strains T and 206. Strain T, shown in fig. 1, has given rise to a slowly but progressively growing tumour in 20 out of 21 mice, whereas with strain 206 (fig. 2) all tumours have finally disappeared spontaneously. These two carcinomatous strains exemplify the possible extremes of type of growth, and the intermediate gradations between them have been actually filled up

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* Bashford, E. F., "The Behaviour of Tumour-cells during Propagation," 'Fourth Scientific Report of the Imperial Cancer Research Fund,' 1911, p. 131.

Exp. T/32 A. All mice inoculated in right axilla with 0·015 c.c. (21.9.10).

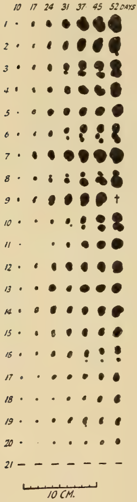

Fig. 1.

Exp. 206/11 C. All mice inoculated in right axilla with 0·02 c.c. (7.1.09).

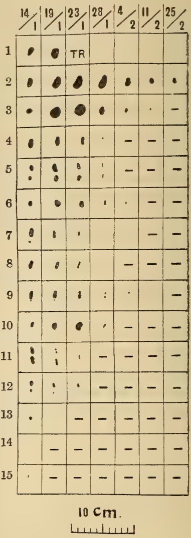

Fig. 2.

Fig. 1.—Chart showing progressive character of the growths developing after implantation of carcinoma T.

Fig. 2.—Chart showing the temporary character of the growth of tumour 206 in normal mice.

in practice in this laboratory by the cultivation of a large number of different tumour strains. There must be one or more factors in constant operation to give the two extreme results cited above, and the understanding of these phenomena has to a considerable extent been advanced by Bashford, Murray, and Haaland,* since they have shown that the resistance to re-inoculation which a tumour-bearing animal frequently exhibits, is of the nature of an active immunity. This development of an active resistance during the growth of a transplanted tumour is a most important factor in determining the character of growth which a tumour will show, and the following experiments will demonstrate that this power of rendering a soil unsuitable for further inoculations is a distinctive feature of various tumour strains.

The procedure adopted has been to inoculate in one flank a series of young animals with a given tumour strain, and then to excise all the growths after 10–30 days, *i.e.* after intervals long enough to allow the tumours to attain a considerable size, 1–4 grm. One, two, or three days after extirpation, the animals have been inoculated on the other flank with a tumour of the same or of another strain. In this way two readings are obtained; the result of inoculating a given series of normal animals, and the result of inoculating the same animals after a tumour had been growing in them over a known period. The precise way in which the experiments have been carried out will be rendered clearer by the accompanying charts, which portray the tumours first inoculated as black silhouettes, whilst the tumours inoculated after operation, as also the controls to the second inoculation, are outlined in black, and filled in with dots.

The experiment with strain T, shown in fig. 3, exemplifies the course of such an experiment conducted with a progressively growing tumour strain. Eleven mice bearing this tumour had their growths excised on the 33rd day, and were re-inoculated with carcinoma 63 two days later. The 11 animals all remained free from recurrence, and in every case strain 63 gave rise to a rapidly and progressively growing tumour. When strain 63 is subjected to a similar analysis, it has been found to give a result similar in every respect to that just shown with strain T. These two carcinomata do not affect the suitability of mice for subsequent grafting, and have been much employed in the present series of experiments, where it was desired to obtain a very accurate estimate of the suitability of a series of mice for transplantation.

The discussion will now be directed to a tumour exhibiting the other extreme type of growth, namely, one which gives rise to temporary growths only. The number of transplantable tumours exhibiting this peculiarity is large, but the date at which spontaneous absorption sets in varies widely from series to series, and from animal to animal, so that the majority of

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* Bashford, E. F., Murray, J. A., and Haaland, M., "Resistance and Susceptibility to Inoculated Cancer," 'Third Scientific Report of the Imperial Cancer Research Fund, 1908, p. 359.

Exp. T/40 C. Mice 1—11 inoculated in right axilla with 0·02 c.c. (20.10.11). Tumours excised (22.11.11), and the animals inoculated in left axilla with 0·02 c.c. of 63/55 E (24.11.11).

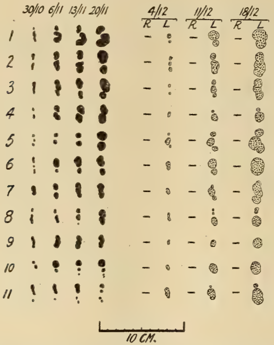

FIG. 3.—The growth of tumour strain T followed by surgical removal does not render mice unsuitable for subsequent inoculation.

these strains are ill-suited for the investigation of resistance. Tumour strain 206 is, however, an exception, as it grows in a very high percentage of inoculated animals, and the date of onset of spontaneous absorption is remarkably regular. Several experiments have been made with this carcinomatous strain, but the results obtained have been so decisive that only one need be illustrated. The result of this experiment is given in fig. 4, where it will be seen that 11 days of growth of this strain in 11 mice has been sufficient to render every one of these animals unsuitable for the growth of carcinoma 63.

Between these two extremes, the other transplantable mouse tumours, carcinomata and sarcomata, range themselves according as they render animals in a higher or lower percentage unsuitable for re-inoculation. Several of these strains have been tested, and resistance to re-inoculation has been found in a percentage varying between 30 and 75. One of these carcinomata, strain 199, exhibits certain peculiarities in its manner of growth, which were described in a communication published in these

Exp. 206/100 B. Mice 1—11 inoculated in right axilla, dose 0·03 c.c. (11.12.11). Tumours excised (22.12.11), and the mice inoculated in left axilla with 0·02 c.c. of 63/56 D (23.12.11). Mice 12—23 : control to re-inoculation.

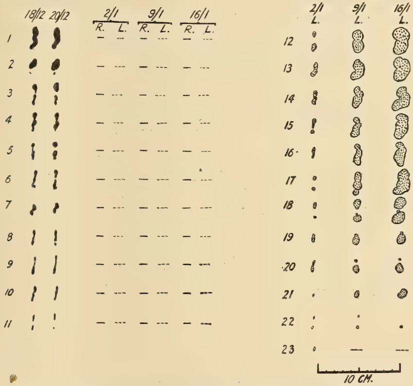

FIG. 4.—Shows the induction of resistance by strain 206 in all mice.

'Proceedings' two years ago.* It was then noted that, in a series of mice inoculated with this strain, about one-third would show progressively growing tumours, another third would show spontaneous absorption after temporary growth of variable duration, whilst the remainder would, after temporary cessation, resume the progressive type of growth. It was also shown that there was an active resistance induced not only in the cases where the tumours had undergone spontaneous absorption, but also where they had temporarily ceased to enlarge their dimensions. The strain still continues to manifest the type of growth observed in the earlier generations. An analysis of the reaction set up by it in a series of mice was attempted by excising the growths on a given day, and then testing the suitability of the animals for re-inoculation. It was found that, in about 60 per cent. of the cases, strain 199 had rendered the animals resistant to re-inoculation, a result which conforms with that previously obtained.

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* Bashford, E. F., and Russell, B. R. G., "Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths," 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 298.

This concludes the description of the findings with mouse tumours, which indicates how extremely variable in action their parenchymata may be, leading from the case where no resistance is induced, through all gradations to the case where resistance is induced in every animal.

The behaviour of a transplantable rat sarcoma, obtained from Jensen, has also been investigated in a manner similar to those already detailed for various mouse tumours. This strain, J.R.S., is a rapidly growing spindle cell sarcoma which gives a high percentage of success on transplantation.

Spontaneous healing occurs with great frequency in series of this tumour, and large masses of growth, weighing 10–15 grm., often disappear entirely. The re-inoculability of rats bearing this tumour has been already described and figured,* and it was then concluded that the results obtained on re-inoculation could be explained only by assuming the development of resistance during the growth of the tumour first inoculated. The presence of large rapidly growing neoplasms during the period in which the re-inoculation tumours are developing, presents both an actual and a theoretical complication which it seemed desirable to eliminate. Accordingly the tumours from the first inoculation have been allowed to develop for a certain period, then all have been excised, and a re-inoculation has been made to ascertain what alteration had taken place in the suitability of the soil.

In one experiment conducted with this rat sarcoma, the result of re-inoculating seven rats, whose tumours had been completely extirpated, was that in only one animal was a tumour obtained approximating those of the control series in the speed of its growth. In two cases the re-inoculation was entirely negative, whilst in the remaining four only small nodules developed. In six of the seven animals there was thus a considerable degree of resistance developed from the tumour first inoculated. In seven other rats from the same series the excision was intentionally incomplete, but the result of re-inoculation was practically the same as in the rats where the excision was complete. These findings do not harmonise with those of Uhlenhuth, Haendel, and Steffenhagen,† who found that incomplete excision left the animals suitable for re-inoculation. In the above series of experiments with rat and mouse tumours, the success or failure of re-inoculation has been found to be determined solely by the nature of the reaction set up by the first implanted tumour.

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* *Loc. cit.*, p. 203.

† Uhlenhuth, Haendel, und Steffenhagen, "Experimentelle Untersuchungen über Rattensarkom," 'Arb. a. d. kais. Gesundheitsamt,' 1911, vol. 36, p. 465.

All of the above experiments have been chosen with the special view of demonstrating how very differently the parenchymata of various tumours of different types behave in regard to the alteration of the suitability of the soil which they induce, and the detailed description of this class of experiments given above will now be followed by a general discussion upon the interpretation which is to be put upon the results obtained.

On the one hand, two tumour strains have been shown, 63 and T, which, in the course of their development, do not alter the suitability of mice for re-inoculation ; on the other hand, a tumour strain has been shown which so alters the animals that all are refractory to subsequent inoculation. It is, of course, apparent that such wide differences can only be attributed to inherent properties of the tumour parenchymata, and the contrast in their behaviour may be drawn by stating that the parenchyma of strain 206 induces a resistance which the parenchymata of 63 and T fail to do. The terminology of modern immunity studies would label the former an efficient antigen, whereas the latter would be inefficient. In these extreme cases the differences are so wide, and the reactions so marked, that the medium, *i.e.* the inoculated mouse, can be regarded as indifferent. When tumours are considered, however, which only induce resistance in a certain percentage of cases, slight differences in the medium turn the scale for or against the inoculated graft in individual cases.

To take the specific instance of strain 199, why does this strain induce resistance in 60 per cent., and fail to do so in the remaining 40 per cent. ? The parenchyma, which has been distributed over 10 mice, for example, although of exactly the same quality and quantity throughout, fails to induce resistance in four mice. Again, in the extreme cases of strains 63 and T, resistance is induced occasionally in a certain number of animals, whilst strain 206 sometimes gives rise to progressively growing tumours in animals exhibiting no reaction of resistance. These variations in the development of resistance in the individuals composing a series must be regarded as the expression of slight differences in the constitution of the animals composing such a series, and, whilst in general the reaction is determined by the tumour parenchyma, a slight individual peculiarity is sufficient at times to determine or prevent the development of resistance. Tumour strains such as 63 and 206 usually mask all individual variations, but strain 199 and many others bring them out with distinctness.

Murray's studies* upon the heredity of cancer have shown that it is possible to breed out families of mice whose members will show an extremely high incidence of cancer, although they are not more suitable for the implantation of a transplantable tumour than mice not specially bred.†

Cuénot and Mercier‡ have made the attempt to breed out from one and the same strain of mice families suitable and unsuitable for the implantation of cancer. They claim to have isolated two families, in one of which a tumour strain will take in 86 per cent., whereas in the other it will only take in 20 per cent. Should these findings be confirmed, and it seems desirable that they should be repeated and tested with a variety of tumours, they would help greatly to explain the character of growth exhibited by such strains as 199. It might be possible to isolate families of mice in which this strain produced no resistance, and the inoculation would lead to the development of progressively growing tumours in all cases.

Variations in the power of tumour parenchymata to induce resistance may be made in part responsible for the adaptations which tumours undergo more especially during their earliest transference to new hosts. When a spontaneous growth is transplanted, there is usually a rapid rise in the percentage of success attached to the first three or four passages. Might it not be possible that the rapid rise in transplantability is due to a greater or smaller loss of the power of the tumour parenchyma to induce resistance? This possibility requires consideration because careful microscopic examination of grafts during the first 10 days shows normal growth in nearly every case, even although the tumour strain only gives in control series an eventual percentage of success of about 40. The natural resistance of animals to tumour inoculation is a phrase which has been much employed, but it might perhaps be more correct to speak of animals which readily develop an active resistance.

The next question to be discussed is, how do the results obtained by re-inoculation after the first tumour is removed compare with those obtained when this growth is not interfered with? It may be stated at once that the results obtained under the two conditions are exactly identical, and the removing or leaving behind of the tumour first inoculated neither favours nor hinders specifically the development of the second one. That a mouse bearing an implanted growth can be successfully re-inoculated was recorded from this laboratory as early as 1904,[*] and more extended researches led to the formulation of the dictum that the better the first tumour grows the more favourable are the chances of the second inoculation being successful.[†] In a paper by Bashford, Murray, Haaland, and Bowen[‡] the conclusion was drawn that negative results on re-inoculation of an animal already bearing a tumour were due to the development of concomitant immunity, and the view was rejected that the negative result was attributable to an exhaustion of specific food-substances (athreptic immunity) by the first tumour. The latter view is still upheld by Apolant,[§] but in one of his later papers the accompanying charts show that after removal of the first tumour the majority of the animals, rats and mice, are resistant to re-inoculation, which is scarcely in harmony with the hypothesis he entertains.

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* Murray, J. A., "Cancerous Ancestry and the Incidence of Cancer in Mice," 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 42.

† Haaland, M., "Spontaneous Cancer in Mice," 'Roy. Soc. Proc.,' B, vol. 83, p. 532.

‡ Cuénot, L., et Mercier, L., "L'hérédité de la sensibilité à la greffe cancéreuse," 'Comptes Rend. de l'Acad. des Sciences,' 1910, vol. 150, p. 1443.

The conclusion is inevitable that tumour parenchymata vary widely in the extent to which they alter the suitability of an animal for growth of a subsequently implanted tumour, and that this alteration of the suitability of the animal is due to the development of an active resistance or immunity.

A clear recognition of differences in the behaviour of transplanted tumours, induction of resistance in a high percentage of cases on the one hand, contrasted with practically total absence on the other, led to a study of the influence which a strain of the former type might have upon the growth of a tumour of the latter type, where both tumours were inoculated at the same time. Simultaneous inoculation of two tumours in opposite axillæ has already been carried out. Bashford, Murray, and Cramer[||] inoculated two separate strains of the same tumour in the right and left axilla respectively for five successive passages, and found that each tumour strain varied in its growth quite independently. Bridré[¶] also performed the double inoculation of two separate strains, and found that each grew as if it alone had been inoculated.

When, however, double inoculation is carried out with tumours of different types of growth, the two tumours do not grow independently of each other.

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[*] Bashford, E. F., and Murray, J. A., "The Transmissibility of Malignant New Growths from one Animal to Another," 'First Scientific Report of the Imperial Cancer Research Fund,' London, 1904, p. 11.

[†] *Loc. cit.*, p. 205.

[‡] Bashford, E. F., Murray, J. A., Haaland, M., and Bowen, W. H., "General Results of Propagation of Malignant New Growths," 'Third Scientific Report of the Imperial Cancer Research Fund,' London, 1908, p. 262.

[§] Apolant, H., "Ueber die Immunität bei Doppelimpfungen von Tumoren," 'Zeitschr. f. Immunitätsforsch.,' 1911, vol. 10, p. 103.

[||] Bashford, E. F., Murray, J. A., Cramer, W., "The Natural and Induced Resistance of Mice to the Growth of Cancer," 'Roy. Soc. Proc.,' 1907, B, vol. 79, p. 164.

[¶] Bridré, J., "Recherches sur le Cancer Expérimental des Souris," 'Ann. de l'Institut Pasteur,' 1907, vol. 21, p. 760.

It has been found that strain 63 is inhibited in its growth by the simultaneous inoculation of sarcoma 37, a tumour which renders mice resistant to re-inoculation in a high percentage of cases. The percentage of success on inoculation of strain 63 has been lowered from 100 down to 50, and even 25, whilst in addition the rate of growth is greatly retarded as compared with that in normal animals.

The effect of a simultaneous inoculation of mouse embryo tissue upon carcinoma 63 has also been studied, but an inhibition of the growth of the tumours has not been observed. It suggests itself as a perfectly legitimate explanation that the inefficacy of the simultaneous inoculation of embryonic tissue to inhibit the growth of tumour 63 is due to the later development of resistance after this treatment, and it may be a question of one or two days only. By using tumour tissue to induce immunity, the immunity can be brought to bear upon the inoculated tumour-tissue of carcinoma 63, before the latter has had time to become fully established. This latter circumstance is of considerable importance, and has been demonstrated by extension of these experiments to other tumour strains. Another carcinoma, strain 91, has been used, but with this tumour it has not yet been possible to demonstrate any inhibition where simultaneous inoculation with sarcoma 37 has been performed. Apparently this tumour cannot be overtaken in its growth by the concomitant immunity arising from the sarcoma, but continues to develop quite as well in animals where the sarcoma is disappearing as it does in the control series.

The behaviour of tumour 63, however, demonstrates very clearly a case where the immunity can overtake the early phases of growth and prevent its continuation. This explanation further accords well with the histological findings in early stages of grafts inoculated in immune animals. Briefly stated, these led to the conclusion that the resistance was directed mainly against the cancer cell's power of inducing a stroma reaction.* The experiments next to be described support the above interpretation of the different behaviour of strains 63 and 91.

The treatment of mice by the inoculation of normal tissues of the mouse, as described in these 'Proceedings,'† has been found to prevent the development of tumours implanted 10 to 20 days later, and the resistance evoked by these normal tissues is of the same nature as the resistance evoked by tumour tissue. The efficacy of this preliminary treatment stands out in marked contrast with the disappointing nature of the results hitherto obtained when the attempt is made to produce, by the same means, the involution of an already established tumour which tends to grow progressively.

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* Russell, B. R. G., "The Nature of Resistance to the Inoculation of Cancer," 'Third Scientific Report of the Imperial Cancer Research Fund,' London, 1908, p. 341.

† *Loc. cit.*, p. 209.

By means of a rather complicated experimental procedure it has been found possible to demonstrate that an immune reaction can be evoked in an animal bearing a progressively growing tumour. The technical difficulties attaching to such an investigation are considerable, for it requires the inoculation of a large number of mice, some of them on two and three occasions, and the preparation of two or even three control series. The inoculated animals require to be kept under observation for a long period, which necessitates the use of a tumour-strain growing rather slowly, and also in a high percentage. Strains which exhibit the phenomenon of concomitant immunisation are quite unsuitable for testing this point. Carcinoma T fulfils all the above conditions, and it is from observations on this tumour that the following conclusions have been arrived at, although subsequently the experiments were repeated with another adeno-carcinoma, strain 91.

On January 19, 1910, 40 mice, weighing from 14 to 16 grm. each, were inoculated with 0·015 c.c. of T/27 C in the right axilla; 29 of the 35 surviving mice developed tumours—83 per cent. Twenty-five of these tumour-bearing mice were divided into two batches, when the tumours were 12 days old, and one batch was inoculated on the back with 0·05 c.c. of mouse carcinoma J, while at the same time 15 normal mice were treated in the same way. Strain J at that time gave rise to temporary proliferation only when an emulsion was inoculated by means of a syringe; the mice in which this temporary growth had taken place became highly refractory, and advantage was taken of this behaviour of the tumour to use it for immunising purposes. Eleven days after this inoculation, and 23 days after the start of the experiment, all these mice were inoculated in the left axilla with 0·015 c.c. of T/28 F, and, in addition, 12 normal mice to serve as an indicator of the transplantability of series T/28 F. The degree of transplantability of this series was found to be 75 per cent.

In the batch which did not receive an immunising dose of tumour J, 10 out of 13 mice developed tumours on re-inoculation; whereas, in the batch which received an intercalated dose of tumour J, only 3 out of 12 mice developed tumours on re-inoculation. In the 15 control animals which were inoculated first with carcinoma J, and then tested with T/28 F, only one developed a tumour from the re-inoculation.

The result of the above experiment may be briefly summarised in percentages in the following way:—

Expt. T/27 C. Success of primary inoculation = 83 per cent.

Re-inoculation of positives.	Re-inoculation of immunised positives.	Re-inoculation of immunised controls.	Control to re-inoculation.
10 in 13 77 per cent.	3 in 12 25 per cent.	1 in 15 7 per cent.	6 in 8 75 per cent.

Three further experiments on these lines were performed with the same strain; the intervals elapsing between the several inoculations were maintained, but the tissue employed to induce the resistance was varied. The behaviour of carcinoma 91 was also tested on two separate occasions.

On summing up the total figures obtained in the six experiments the change induced can be seen, as in the following totals:—

Re-inoculation of positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to re-inoculation.
41 in 65 63 per cent.	19 in 70 27 per cent.	22 in 95 23 per cent.	51 in 69 73 per cent.

Whereas 63 per cent. of mice bearing tumours of these two strains have been shown to be receptive to a second inoculation, this figure is reduced to 27 per cent. when the second inoculation is preceded some 14 to 16 days by the injection of an immunising dose of tissue. The figures are too large to allow the simple interpretation of the results as the expression of an involuntary selection. The percentages given in Columns 2 and 3, which deal with the "immune reaction" in tumour-bearing mice and in normal mice respectively, show that, in general, mice with tumours can be rendered resistant with almost the same facility as normal mice.

When carrying out the experiments upon the immunisation of mice bearing tumours, described in full in the preceding pages, some cases were noted where the tumour, which had already started growth before the immunisation was carried out, was retarded greatly in its growth, or even totally inhibited. If this result could be regularly obtained, the cure of transplanted tumours which did not disappear spontaneously would be accomplished. Strains of tumours which grow progressively were tested by subjecting the mice bearing them to the inoculation of embryonic tissue or of tumour tissue, which disappeared spontaneously. The effect of single inoculations of varying amounts of immunising tissue was tested, and also the repetition of the immunising dose at various intervals of 7, 10, and

14 days, in the hope that in this way a high degree of immunity might be maintained over a long period.

The result obtained was not encouraging, even although the strains employed fell a ready prey to a resistance evoked before grafting. Taken over all, the tumours grew more slowly than in the control animals, but their regression was not brought about.

The main conclusions to be drawn from the above investigations are :—

1. Tumour parenchymata vary widely in their power of inducing resistance. This factor must not be lost sight of in the prosecution of researches in immunity to transplanted cancer.

2. The individuality of the animal inoculated may contribute to the development of resistance, although not to so marked a degree as the tumour parenchyma.

3. Simultaneous inoculation of a tumour strain which induces no resistance, and a strain which induces resistance, may be followed by marked inhibition of the growth of the former strain.

4. Mice bearing progressively growing tumours can be rendered resistant to re-inoculation, but the tumour first inoculated need not necessarily be affected.

5. Repeated inoculations of tissues, such as mouse embryo skin which renders animals resistant to subsequent inoculation, have not been shown to have a constant effect upon the growth of established tumours.

6. The conclusions drawn in (4) and (5) support the view previously expressed that immunity to cancer is directed mainly against the stroma-eliciting properties of the cancer cell.

The Distribution of Oxydases in Plants and their Rôle in the Formation of Pigments.

By Frederick Keeble, Sc.D., Professor of Botany, University College, Reading, and E. Frankland Armstrong, Ph.D., D.Sc.

(Communicated by W. Bateson, F.R.S. Received March 15,—Read May 2, 1912.)

Whereas our knowledge of the mode of inheritance of flower colour has made rapid and sure advance of recent years, our understanding of the chemistry of the process of pigment formation in flowers is still uncertain and incomplete.

The researches of Miss Wheldale (1911) on flower-pigments and of Gortner and others on animal-pigments have confirmed and extended the conclusions of earlier workers, and it may now be regarded as an established fact that the formation of pigment in plants and in animals is due to the action of an oxydase on a more or less colourless chromogen.

According to the experiments of Gortner (1911) the superficial pigmentation of various insects is the outcome of the action of tyrosinase on the chromogen tyrosin. As a result of that action a black pigment, melanin, is produced. Gortner has shown moreover that this reaction may be inhibited by certain phenolic compounds such as phloroglucinol, orcinol and resorcinol. These dihydroxyphenols Gortner describes as anti-enzymes and he attributes the phenomenon of dominant whiteness, that is the suppression of pigment-formation in tissues, believed, by reason of their mode of colour-inheritance, to contain both chromogen and oxydase, to the presence—local or general—of these anti-bodies.

Miss Wheldale has obtained evidence that pigment formation in flowers is consequent upon the interaction of an oxydase and a chromogen and has suggested that the latter body is a product of hydrolysis of a glucoside.

Pending the introduction of more precise and convenient methods for determining the distribution of oxydases in plant tissues our knowledge has remained in this interesting but incomplete state. It is true that a number of oxydase reagents is known but the method of their application, that of adding the reagents to macerated tissues, cannot but lead to inconclusive results. It is possible, however, as Clarke (1911) has demonstrated, to use certain oxydase reagents for micro-chemical purposes and, as we show, appropriate methods admit of both macroscopic and microscopic demonstration not only of the presence but also of the distribution of oxydases in such delicate tissues as the petals of flowers.

Further, as will be apparent to all students of Genetics, the application of precise chemical methods to the investigation of the distribution of oxydases in plant- and animal-tissues is of special importance at the present time. For, thanks to the work of Bateson, Baur, Gregory and many others, not only do we possess a detailed knowledge of the modes of inheritance of flower-colour in many different species of animals and plants, but we have also at our disposal many pure-bred strains of animals and plants, the genetical relationships of which are known. Therefore we may hope that precise tests applied to plants or animals of known genetical constitution may throw new light on the physiological *rôles* of oxydases in the organism and may contribute also to an understanding of the nature of Mendelian characters, the sum total of which appears to determine specific character.

The method which we employ consists in the treatment of a tissue with the colour-indicator constituent of the oxydase reagent together with a "hormone" (see H. E. and E. F. Armstrong, 1910, 1911), that is a substance which causes the plasmatic membrane to become permeable to the reagent and also renders active the oxydases and other enzymes present in the cell. When this component of the oxydase reagent is added to the intact petals of a flower the first visible effect is the decolorisation of the flower. As soon as the colourless state has been induced, the second component of the reagent, hydrogen peroxide, is added. Owing to the previous treatment of the tissues the hydrogen peroxide penetrates rapidly into the cells and the colour-reaction indicative of oxydase is produced, generally in the course of a few minutes. By the use of various oxydase-indicators, α-naphthol, benzidine and others, we have been enabled to establish the following facts with respect to (1) the distribution of oxydases in the Chinese primrose (*Primula sinensis*) and (2) the relation between oxydases and pigment formation in that plant:—

1. The distribution of pigment in the flower coincides exactly with that of a peroxydase.

2. The alcoholic solution of the oxydase-indicator brings about decolorisation of the flower. In most cases the decolorisation is rapid and complete; but in certain colour-varieties the flower-colour is more resistant. Surface sections of such flowers may however be readily decolorised. The colourless state is maintained till hydrogen peroxide is added. Hence the oxydising agents present in the flower in *Primula sinensis* are to be regarded as peroxydases.

3. The use of different oxydasic reagents reveals the presence of two

peroxydases in *P. sinensis.* One, the "epidermal" peroxydase, occurs in the epidermis and in some cases in the layers subjacent to the epidermis. Another, the "bundle" peroxydase, is localised in layers of cells neighbouring the woody tissues of the vascular bundles.

Both epidermal and bundle peroxydases occur in the vegetative parts of the plant as well as in the flower. In the stem the epidermal peroxydase is separated widely from the bundle peroxydase by many layers of cortical cells, which in many varieties at all events contain no peroxydase; but in the tenuous petals of the flower the bundle peroxydase occurs of necessity in close proximity with the epidermal peroxydase.

4. Certain varieties of *P. sinensis* such as Crimson King, Coral Pink, and Sirdar give, under certain circumstances, a *direct* oxydase reaction: that is a characteristic coloration is produced when they are treated with the oxydase reagent alone.

5. The bundle peroxydase of the petals of the flower of *P. sinensis* is located in the cells of the bundle sheath which surrounds the veins. The peroxydase accompanies that sheath throughout the repeated ramification of the veins, and may be seen in microscopic preparations to extend to the tips of their finest branches; the epidermal peroxydase occurs in the superficial papillate cells of the petals.

6. Where, as is the case with many varieties of *P. sinensis,* the flower has a yellow eye, no epidermal peroxydase is demonstrable over the eye except in the hairs which are produced as outgrowths from the epidermis.

7. The epidermal and bundle peroxydases differ from one another both in their distribution and in their colour reactions. Thus bundle peroxydase reacts with α-naphthol and hydrogen peroxide to yield a lavender-blue colour which picks out the veins in exquisite detail. With this reagent the epidermal peroxydase yields generally no colour reaction.

Selective coloration in the opposite sense though less precise is produced by the addition of benzidine and hydrogen peroxide to the flower. Treatment with an alcoholic solution of benzidine brings about first decolorisation of the sap (anthocyan) pigments of shoot and flower. The subsequent addition of hydrogen peroxide activates both epidermal and bundle peroxydases and results in a rich brown uniform coloration of the surface layer of the petals, which coloration extends also to the veins.

8. White flowers which by breeding tests are known to be dominant whites fail to give the epidermal peroxydase reaction; but in such flowers a faint bundle peroxydase reaction may occur.

Both epidermal and bundle peroxydases are, however, present in dominant white flowers; for if such flowers are treated with hydrogen cyanide and subsequently with benzidine or α-naphthol and hydrogen peroxide the characteristic peroxydase reactions are produced. The whole surface of the petals becomes deeply coloured and the veins also stand out prominently. Hence the flowers of dominant white primulas contain a substance which inhibits but does not destroy the pigment-producing peroxydase.

Where dominant white patches occur on otherwise self (uniformly) coloured flowers, as is the case with certain strains of blue *P. sinensis* with which we have worked, the benzidine reagent picks out the coloured areas in brown and leaves the dominant white areas uncoloured. The α-naphthol reagent picks out the veins with the utmost sharpness and leaves the dominant white patches unstained except for an occasional fine line of colour along the course of a vein which traverses the white patch. Beyond each dominant white area as well as before reaching it, the bundle sheath surrounding each vein is more deeply stained. Recessive whites possess both epidermal and bundle peroxydases.

9. The observations on the epidermal and bundle peroxydases throw light on the significance of the phenomenon presented by many cultivated flowers which are known to florists as "ever sporting." Such flowers are characterised by the sporadic appearance of splashes of colour on a white or self-coloured ground. Ever sporting strains are familiar to everybody in carnations and azaleas. In *P. sinensis* ever sporting strains are also common. Thus the variety Mont Blanc Star bears white flowers with magenta flakes. We are inclined to regard flaking as the effect of the bundle peroxydase on the chromogen-containing cells neighbouring on the bundle sheath. The white ground colour is to be attributed to an inhibitor associated with and nullifying the epidermal peroxydase.

The marked localisation of the pigmentation effected by the bundle peroxydase, which localisation expresses itself in splashes, flakes, or lines of colour, appears to be due to anatomical causes, such as the degree of development of the cells of the bundle sheath and the nearness of the veins to one another.

10. The existence of two localised peroxydases which may induce pigmentation and may reinforce one another along certain tracts of tissue provides material facts for the explanation of colour-range and colour pattern in flowers.

The investigations which are the subject of this communication have been carried out jointly by the authors, who wish to share equally in the responsibility for the results which they have obtained.

The cost of the research, a detailed account of which will be published elsewhere, has been defrayed in part by grants from the Royal Society and from the British Association.

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On the Nature of Pancreatic Diabetes. (Preliminary Communication.)

By F. P. Knowlton and E. H. Starling, F.R.S.

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(From the Institute of Physiology, University College, London.)

Many explanations have been proposed for the fact, discovered by von Mering and Minkowski (1), that extirpation of the pancreas is followed immediately by severe and fatal diabetes. It has been suggested on the one hand that the normal function of the pancreas is to diminish excessive production of sugar, and that, in the absence of its restraining influence, excessive sugar production and mobilisation are the results. On the other hand, the fact that carbohydrates are not utilised by the body when administered to animals in this condition has been interpreted as showing that the tissues have lost their normal power of assimilating and utilising glucose. It has also been suggested, though without much experimental support, that the sugar of the blood has to be built up into some other form before it can be utilised by the tissues.

We have recently, in a research on the influence of mechanical conditions and of temperature on the heart beat, modified the procedure described by Jerusalem and Starling (2) for working with a heart-lung preparation, so that we are able to keep a heart, connected with the lungs but isolated from the rest of the body, beating for many hours in approximately normal conditions, *i.e.* working at a normal arterial pressure and with a normal output. In this preparation we are able to vary at will the venous filling of the heart, the arterial resistance, or the temperature. The total amount of blood employed is about 300 c.c., and as the heart of a small dog puts out about 150 to 250 c.c. of blood per minute, the whole of the blood in the apparatus circulates through the heart once in every two minutes. It occurred to us that it might be possible by using this preparation to throw light on the pathogeny of pancreatic diabetes.

I. *The Consumption of Sugar in the Normal Heart.*

The power of the tissues to utilise glucose directly was asserted by Chauveau and Kaufmann (3) in the case of voluntary muscle, and has been proved in the case of the heart transfused with Ringer's fluid, by Locke and Rosenheim (4), and by Rohde (5) and others. In our experiments, a small proportion of glucose having been added to the blood, it was allowed to circulate through the heart at a temperature of 37° C. continuously for two to three hours. The arterial pressure was kept throughout at 80 to 90 mm. Hg. The following table represents some of the results obtained:—

Consumption of Sugar by the Heart.

A. Normal Heart Fed with Normal Blood.

	First period (1 hr.).			Second period (1 hr.).		
	Sugar in blood.		Milligrammes sugar per grm. heart.*	Sugar in blood.		Milligrammes sugar per grm. heart.*
	Before.	After.		Before.	After.	
	per cent.	per cent.		per cent.	per cent.	
Dog	0·79	0·66	5·8	0·66	0·59	5·6
	0·70	0·61	4·8	0·61	0·56	3·5
	0·20	0·11	3·9	0·32	0·25	3·5
Cat	0·58	0·51	4·2			
	0·72	0·65	5·1			
	Sugar in serum.			Sugar in serum.		
	per cent.	per cent.		per cent.	per cent.	
Dog	0·63	0·50	4·1	0·50	0·33	4·1
	0·81	0·69	3·5	0·69	0·57	2·8
	0·89	0·71	5·15			

* This column was calculated after determining the total volume of blood in circulation and the weight of the heart.

From these results we may conclude that the normal heart fed with normal blood under approximately physiological conditions consumes about 4 mgrm. of sugar per hour and per gramme of heart muscle.

II. *The Consumption of Sugar in the Diabetic Heart.*

Similar experiments were carried out on the hearts of dogs from which the pancreas had been removed from three to six days previously. The following table represents the results obtained in six such experiments:—

Sugar Consumption in Diabetic Heart.

Sugar in serum.		Milligramme sugar per grm. heart per hour.
Before.	After 1 hr.	
per cent.	per cent.	
0·59	0·60	0
0·30	0·24	1·1
0·74	0·74	0
0·57	0·54 (1½ hrs.)	1·0
0·53	0·52	0·5
0·50	0·49	0·5

It will be seen that the consumption of sugar was very much less than in normal hearts. In two cases the sugar titration gave the same results at the beginning and at the end of the hour. In two other cases a difference in titration was obtained which pointed to a consumption of sugar of 0·5 mgrm. per gramme of heart muscle.

But this difference was within the limits of our experimental error. A series of parallel estimations of sugar in different samples of serum gave the following results:—

Blood	1.	2.	3.	4.	5.
	per cent.	per cent.	per cent.	per cent.	per cent.
Sample A	0·63	0·61	0·365	0·36	0·37
„ B	0·62	0·62	0·37	0·38	0·37

The average experimental error was therefore 0·01 per cent., and the *maximal* error 0·02 per cent. Moreover, we have evidence of the occurrence of a certain small amount of glycolysis in the blood itself. This in normal blood was probably not greater than 0·01 per cent. per hour during the first two hours of the experiment. In diabetic blood taken at the end of the experiment a somewhat higher degree of glycolysis was occasionally observed.

The following results give the glycolysis actually observed in the blood itself:—

Percentage of Sugar.			Glycolysis per hour.
A. Normal blood + dextrose—			
1st sample.	2nd sample (after 1½ hrs. at 37° C.).	3rd sample (after 3 hrs. at 37° C.).	
A 0·62	0·62	0·59	0·01 per cent.
B 0·63	0·61		
B. Diabetic blood alone—			
1st sample.	2nd sample (after 1½ hrs. at 37° C.).		
A 0·22	0·175 (+ pancreatic extract)		0·025 „
B 0·22	0·18 (+ saline solution)		
C. Diabetic blood + dextrose (after use in experiment)—			
(1) 1st sample.	2nd sample (after 1 hr.).		
A 0·36	0·37		0·00 „
B 0·38	0·37		
(2) 1st sample.	2nd sample (after 3 hrs.).		
0·33	0·24		0·03 ,
(3) 1st sample.	2nd sample (after 2 hrs.).		
0·91	0·89		0·01 „

It is possible that a summation of glycolysis and experimental error would account for the highest figures obtained in the series of experiments on the diabetic heart. They show at any rate that the consumption of sugar by the diabetic heart is minimal or, as we are inclined to believe, entirely absent.

III. *Is this loss of the Power to utilise Sugar in the Diabetic Heart due to the Absence of some Substance normally present in the Heart and Circulating Blood and presumably formed by the Pancreas, or to the Presence of some Toxic Substance accumulating as a result of Extirpation of the Pancreas and preventing the Utilisation of Sugar by the Tissues?*

Two sets of experiments point to the former explanation as the most probable. In the first set of experiments the heart from a diabetic animal was fed with blood from a normal animal. In this case the consumption of sugar, even in the first hour, was considerably above that observed in the diabetic hearts fed with diabetic blood, and steadily increased during the next two hours of the experiment.

Consumption of Sugar by Diabetic Heart Fed with Blood from a Normal Dog.

Milligrammes per grm. Heart Muscle.

1st hour.	2nd hour.	3rd hour.
2·9	5·28	8·1

In the reverse experiment a normal heart was fed with diabetic blood. In this case the consumption of sugar during the first hour was only slightly below normal and steadily diminished during the succeeding two hours, as is shown by the following figures:—

1st hour.	2nd hour.	3rd hour.
3·5	2·5	1·7

The obvious interpretation of these two sets of experiments is that the tissues and blood normally contain some substance, the presence of which is essential for the direct utilisation of sugar by the tissues. This substance is gradually used up in the tissues, and therefore has to be continually replaced from the blood if the utilisation of sugar is to continue.

IV. *Is this Substance derived from the Pancreas?*

To answer this question an extract of pancreas was made by grinding up the gland, boiling it in slightly acid Ringer's solution, and filtering it to free it from the coagulated proteins; 10 c.c. of the filtrate were neutralised with a few drops of sodium carbonate solution and added to the blood circulating through the heart of a diabetic animal. The addition was in each case made during the second hour of the experiment, in order that the consumption of sugar by the heart during the first hour might be tested. The following results were obtained:—

Diabetic Heart Fed with Diabetic Blood.

Consumption of Sugar in milligrammes per grm. of Heart Muscle.

1st hour (blood alone).	2nd hour (after addition of pancreatic extract).
1·5	4·3
0·5	3·0
0·5	2·8
0·5	3·6

It will be seen that in every case the addition of a boiled extract of pancreas, whether derived from the same animal (dog) or from another animal (cat), raised the sugar consumption by the heart from a figure which was within the limits of experimental error to a point closely approaching that found in normal hearts. The effect of the pancreatic extract in improving the action of the heart was evident during the experiment itself. In most cases the heart in the diabetic animal, under the conditions of the experiment, has a lower rate of beat than that found in the normal animal. The effect of adding the extract of pancreas was to quicken the rate of beat up to a normal figure. Thus, in one experiment, the rate of beat during the first hour was 108 per minute, while feeding with diabetic blood; during the second hour, after the addition of pancreatic extract to the same blood, the heart beats rose to 144 per minute.

We hope to continue these experiments, and especially to determine the respiratory quotient in the normal and in the diabetic heart, and the influence of pancreatic extract on the same. So far as our results go, they seem to indicate that the pancreas normally produces a hormone which circulates in the blood, and the presence of which is necessary in order that the tissue cells may be able to assimilate and utilise the sugar of the blood. In fact, they indicate that the second of the two explanations, which have been mentioned above as having been proposed for the occurrence of pancreatic diabetes, is essentially correct.

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The Measurement of Trypanosoma rhodesiense.

By J. W. W. Stephens, M.D. Cantab., D.P.H., and H. B. Fantham, D.Sc. Lond., B.A. Cantab.

(Communicated by Sir Ronald Ross, K.C.B., F.R.S. Received March 28,—Read May 2, 1912.)

[Plate 3.]

Introduction.

The following paper contains the results of a biometric study of *Trypanosoma rhodesiense* (Stephens and Fantham).

This trypanosome, which is dimorphic, was described by us in July, 1910. It was considered to be a new species of trypanosome, producing Sleeping Sickness in man, since it could be distinguished morphologically by the fact that a certain percentage of short forms showed the nucleus either close to or even posterior to the blepharoplast, a feature which has never been recorded for *T. gambiense*, either before or since.

Otherwise, in *external* morphology *T. rhodesiense* closely resembles *T. gambiense*, for there are long, slender forms and short, stumpy forms, together with intermediate forms. These flagellates were figured by us

(1910) in our original plate and are well shown in the accompanying coloured plate, for which we are indebted to Lady Bruce.

Methods.

The blood-films used were quickly dried, fixed in absolute alcohol, and stained with a modified Romanowsky solution. Films of this nature contain trypanosomes most nearly approximating to the natural size. The flagellates suffer shrinkage in films fixed with sublimate-alcohol.

One thousand specimens of the trypanosome have been measured after the manner introduced by Sir David Bruce for the differentiation of various trypanosomes. In this method the length of the median longitudinal axis, including the free flagellum, is determined as accurately as possible. We found it advisable to modify Bruce's method in some respects:—

(1) Instead of drawing the trypanosomes with a camera lucida, it is much easier to project them on a screen, using a photomicrographic apparatus in a dark room, and then to trace them in outline with a finely pointed pencil. The magnification is verified by projecting a millimetre scale in the same manner. The magnification adopted was 2500 diameters, using a 2-mm. apochromatic objective and an 8 compensating ocular. This method not only saves much eyestrain in drawing, but is also much quicker.

(2) A more important modification consists in the actual mode of measuring the trypanosomes drawn on paper. Sir David Bruce uses for this purpose a pair of compasses, set at a fixed distance of 2 mm., his trypanosomes being magnified 2000 times. There are, however, two objections to this method:—

(*a*) It cannot and does not give an accurate measurement, because the compass makes a series of "jumps" and theoretically and actually the measurements given are always less than the true ones.

We can illustrate our objections perhaps by supposing that we have to measure the outline made by the teeth of a saw. If the teeth are equal and the distance between the compass-points is equal to the depth of a tooth, then the course can be measured. If the depths of the teeth are unequal, then it will be impossible to get an accurate measurement by the compass method, though this can be accurately done by the "tangent line" method. Although the curves of a trypanosome do not change their direction so acutely as the outline of a saw, yet the curves often do change their direction to some extent and the principle of the objection remains. We therefore used the method which we call the "tangent line" method.

The requirements are:—(1) a piece of tracing paper on which a straight line is drawn in ink, (2) a pin, (3) a millimetre scale. The tracing paper is placed over the drawing of the trypanosome, which is seen through it. When the tracing paper is fixed by slight pressure of the pin placed on the ink line, the tracing paper can be rotated and the most tortuous curves followed with ease. One end of the ink line is placed on one end of the trypanosome. If the axis of the trypanosome curves, for example, at the nucleus, the pin is placed at this point and the paper is now rotated until the ink line coincides with the new direction of the axis. This is done as often as is necessary, and in fact the sharpest curves can be followed in this way, which is impossible by a compass, the points of which are at a fixed distance. Finally the other end of the trypanosome is reached, the pin is placed there and the actual extent of the ink line traversed is measured by the millimetre scale. Further, the method has the advantage that it can equally well be applied to the measurement of any other curved line, for example, the axis of a spirochaete.

(*b*) Another objection to the compass method is that, if a start be made at the non-flagellar end of the trypanosome, it is uncertain that the finish will be exactly at the end of the flagellum. If not, there is always a portion of a compass distance which has to be guessed. With the tangent line method this is avoided, and the finish is exactly at the end.

The measurements could also be made by a self-registering rotameter ("map-measurer"), but we think that it is not quite such a convenient method for accurately following the curve.

It may be added that all the trypanosomes were outlined by one of us, and measured by the other.

Measurements and Results.

The following table gives the distribution, in respect to length, of 1000 specimens of *T. rhodesiense* taken from various hosts, and measured in groups of 20 consecutive trypanosomes, neglecting only dividing forms.

Table I.—Distribution in respect to Length of 1000 Individuals of *Trypanosoma rhodesiense*.

Animal.	In microns.																												Average length.
	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.	
Man	—	—	—	—	—	1	—	2	3	4	2	1	1	1	3	1	—	1	—	—	—	—	—	—	—	—	—	—	22·4
„	—	1	—	1	3	—	2	2	3	—	1	2	1	—	2	1	1	—	—	—	—	—	—	—	—	—	—	—	20·4
„	—	—	—	—	—	1	2	—	—	4	5	3	2	2	—	—	1	—	—	—	—	—	—	—	—	—	—	—	22·1
„	1	—	1	1	—	3	3	2	2	2	—	1	—	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	19·8
„	—	—	—	1	—	—	2	3	—	2	1	—	4	2	3	1	—	—	—	1	—	—	—	—	—	—	—	—	22·7
Monkey	—	—	—	—	—	1	—	2	3	1	3	—	2	2	3	—	2	—	1	—	—	—	—	—	—	—	—	—	23·2
„	—	—	—	1	1	—	2	1	2	3	2	—	3	2	2	1	—	—	—	—	—	—	—	—	—	—	—	—	21·7
Horse	—	—	1	—	—	—	1	1	3	—	—	—	—	—	2	—	4	1	6	—	1	—	—	—	—	—	—	—	25·8
„	—	—	1	—	1	1	3	1	1	—	2	—	1	3	1	1	4	—	—	—	—	—	—	—	—	—	—	—	22·4
Dog	—	—	2	—	2	—	4	2	4	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18·9
„	—	—	—	—	2	—	—	3	9	1	—	1	1	—	1	—	2	—	—	—	—	—	—	—	—	—	—	—	20·9
Rabbit	—	—	—	—	—	—	2	1	4	1	2	4	4	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	22·0
„	—	—	1	6	2	3	5	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16·8
Guinea-pig 25	—	—	—	—	—	1	—	—	—	—	3	1	—	2	2	1	5	1	3	1	—	—	—	—	—	—	—	—	26·2
„ 25	—	—	—	—	—	2	1	—	1	2	—	—	2	—	1	1	5	1	4	—	—	—	—	—	—	—	—	—	25·2
„ 21	—	—	—	—	—	—	1	—	3	1	—	1	4	2	2	3	2	—	—	1	—	—	—	—	—	—	—	—	24·4
„ 24	—	—	—	1	3	—	3	2	2	—	—	1	—	—	4	2	1	—	1	—	—	—	—	—	—	—	—	—	21·7
„ 24	—	—	—	—	—	—	2	1	2	—	1	2	4	—	1	1	3	1	2	—	—	—	—	—	—	—	—	—	24·2
Mouse A	—	—	—	—	—	1	3	1	4	1	3	3	1	—	2	—	—	1	—	—	—	—	—	—	—	—	—	—	21·5
„ B	—	—	1	—	1	1	1	3	4	—	5	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20·4
Rat B 16	—	—	—	1	—	—	—	2	3	2	3	1	2	2	3	—	1	—	—	—	—	—	—	—	—	—	—	—	22·4
„	—	—	—	—	—	1	1	1	2	3	5	2	1	2	—	2	—	—	—	—	—	—	—	—	—	—	—	—	22·0
„	—	—	—	—	—	—	—	—	1	1	1	—	—	—	4	—	—	3	2	4	1	2	1	—	—	—	—	—	28·5
„	—	1	2	2	3	3	2	3	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17·2
„	—	—	—	2	2	1	3	1	5	3	—	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19·4
„	—	—	—	—	—	—	—	2	—	—	1	4	1	2	1	1	3	3	1	1	—	—	—	—	—	—	—	—	25·5
„	—	—	—	—	—	1	—	—	3	1	1	1	—	—	3	1	3	2	3	1	—	—	—	—	—	—	—	—	25·5
„	—	—	—	—	1	—	1	—	—	2	2	3	2	1	5	—	1	1	1	—	—	—	—	—	—	—	—	—	23·9
„	—	—	—	—	—	—	—	2	1	2	—	—	2	—	4	5	1	—	1	1	1	—	—	—	—	—	—	—	25·4
„	—	—	—	—	—	—	2	—	4	—	2	4	1	2	2	1	1	1	—	—	—	—	—	—	—	—	—	—	23·1
„	—	—	—	1	—	1	—	1	—	—	3	—	3	—	6	1	1	3	—	—	—	—	—	—	—	—	—	—	24·3
„	—	1	1	1	2	3	1	3	2	2	1	—	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	19·0

Rat B 40.........	—	—	—	—	—	—	—	—	—	1	—	1	3	1	3	2	3	3	2	—	—	1	—	—	—	—	—	—	26·8
"	—	—	—	—	—	—	—	—	—	—	—	2	—	2	—	8	2	2	2	2	—	—	—	—	—	—	—	—	27·4
Rat B 41.........	—	—	—	—	—	—	1	—	—	—	1	3	1	3	—	3	—	1	4	—	1	1	1	—	—	—	—	—	26·8
"	—	—	—	—	—	—	—	—	—	—	1	—	3	2	2	—	2	1	4	4	—	1	—	—	—	—	—	—	27·9
Rat B 42.........	—	—	—	—	—	—	—	—	—	—	2	—	1	—	2	2	—	4	2	3	1	3	—	—	—	—	—	—	28·7
"	—	—	—	—	—	—	—	—	—	—	1	2	—	3	—	2	2	1	1	4	1	1	—	1	1	—	—	—	28·6
"	—	—	—	—	—	—	—	—	—	—	—	1	2	1	2	1	1	1	2	2	3	3	1	—	—	—	—	—	29·1
"	—	—	—	—	—	—	1	1	4	1	1	1	—	1	2	2	2	2	1	1	—	—	—	—	—	—	—	—	24·4
Rat B 34.........	—	—	—	—	—	2	—	—	2	—	3	1	—	—	—	1	3	5	2	—	1	—	—	—	—	—	—	—	25·5
"	—	—	—	—	—	—	3	—	—	1	1	—	3	—	—	—	3	4	1	—	1	1	1	—	—	—	—	1	26·8
"	—	—	—	—	—	—	1	1	—	2	1	1	1	1	2	1	2	1	2	1	2	—	1	—	—	—	—	—	26·3
"	—	—	—	—	2	1	3	—	2	—	—	2	—	2	—	2	1	2	2	1	—	—	—	—	—	—	—	—	23·5
Rat B 46.........	—	—	—	—	—	1	4	2	—	—	2	2	2	3	1	2	1	—	—	—	—	—	—	—	—	—	—	—	22·4
"	—	—	—	—	1	1	1	4	2	—	2	—	—	3	3	3	—	—	—	—	—	—	—	—	—	—	—	—	22·2
"	—	—	—	—	—	1	1	1	—	1	4	—	3	2	1	2	2	1	1	—	—	—	—	—	—	—	—	—	24·0
"	—	—	—	—	1	2	2	1	2	1	—	1	—	3	—	2	4	1	—	—	—	—	—	—	—	—	—	—	22·9
"	—	—	—	—	—	—	1	—	3	3	1	2	1	2	3	1	2	1	—	—	—	—	—	—	—	—	—	—	23·6
"	—	—	—	1	2	2	2	1	1	—	1	1	1	1	5	—	1	1	—	—	—	—	—	—	—	—	—	—	21·8
Total	1	3	10	19	29	35	67	54	92	51	74	56	68	59	85	61	72	50	52	28	13	13	5	1	1	—	—	1	
Percentages	0·1	0·3	1·0	1·9	2·9	3·5	6·7	5·4	9·2	5·1	7·4	5·6	6·8	5·9	8·5	6·1	7·2	5·0	5·2	2·8	1·3	1·3	0·5	0·1	0·1	—	—	0·1	

In the following table the foregoing data are summarised to show the average, maximum and minimum lengths in the different hosts on various days of infection.

Table II.—Measurements of the Length of *Trypanosoma rhodesiense.*

Animal.	Day of infection.	In microns.		
		Average length.	Maximum length.	Minimum length.
Man	80 approx.	22 ·4	29 ·0	17 ·0
,,	117 ,,	20 ·4	28 ·0	13 ·0
,,	117 ,,	22 ·1	28 ·0	17 ·0
,,	123 ,,	19 ·8	30 ·0	12 ·0
,,	123 ,,	22 ·7	31 ·0	15 ·0
Monkey	9	23 ·2	30 ·0	17 ·0
,,	10	21 ·7	27 ·0	15 ·0
Horse	31	25 ·8	32 ·0	14 ·0
,,	32	22 ·4	28 ·0	14 ·0
Dog	4	18 ·9	22 ·0	14 ·0
,,	7	20 ·9	28 ·0	16 ·0
Rabbit	25	22 ·0	27 ·0	18 ·0
,,	38	16 ·8	20 ·0	14 ·0
Guinea-pig 25	18	26 ·2	31 ·0	17 ·0
,, 25	18	25 ·2	30 ·0	17 ·0
,, 21	43	24 ·4	31 ·0	18 ·0
,, 24	56	21 ·7	30 ·0	15 ·0
,, 24	58	24 ·2	30 ·0	18 ·0
Mouse A	6	21 ·5	29 ·0	17 ·0
,, B	7	20 ·4	25 ·0	14 ·0
Rat B 16	4	22 ·4	28 ·0	15 ·0
,,	5	22 ·0	27 ·0	17 ·0
,,	6	28 ·5	34 ·0	20 ·0
,,	7	17 ·2	22 ·0	13 ·0
,,	8	19 ·4	25 ·0	15 ·0
,,	9	25 ·5	31 ·0	19 ·0
,,	10	25 ·5	31 ·0	17 ·0
,,	11	23 ·9	30 ·0	16 ·0
,,	12	25 ·4	32 ·0	19 ·0
,,	12	23 ·1	29 ·0	18 ·0
,,	13	24 ·3	29 ·0	15 ·0
,,	13	19 ·0	27 ·0	13 ·0
Rat B 40	3	26 ·8	33 ·0	21 ·0
,,	3	27 ·4	31 ·0	23 ·0
Rat B 41	3	26 ·8	34 ·0	18 ·0
,,	3	27 ·9	33 ·0	22 ·0
Rat B 42	7	28 ·7	33 ·0	22 ·0
,,	7	28 ·6	36 ·0	22 ·0
,,	7	29 ·1	34 ·0	23 ·0
,,	7	24 ·4	31 ·0	18 ·0
Rat B 34	11	25 ·5	32 ·0	17 ·0
,,	11	26 ·8	39 ·0	18 ·0
,,	11	26 ·3	34 ·0	18 ·0
,,	11	23 ·5	31 ·0	16 ·0
Rat B 46	12	22 ·4	28 ·0	17 ·0
,,	12	22 ·2	27 ·0	16 ·0
,,	12	24 ·0	30 ·0	17 ·0
,,	12	22 ·9	29 ·0	16 ·0
,,	12	23 ·6	29 ·0	18 ·0
,,	12	21 ·8	29 ·0	15 ·0
		23 ·6	39 ·0	12 ·0

On comparing these results with those obtained by Sir David Bruce for 1000 *T. gambiense* and 1000 *T. brucei* respectively, we get the following results:—

	In microns.		
	Average length.	Maximum length.	Minimum length.
T. rhodesiense	23·6	39	12
T. gambiense	22·1	33	13
T. brucei	23·2	38	13

From this table it is seen that the measurements of *T. rhodesiense* are practically the same as those of *T. brucei*, but differ from those of *T. gambiense*.

The average length of *T. rhodesiense* in man and other species of animals, summarised from Table I, is as follows:—

Table III.

Animal.	In microns.		
	Average length.	Maximum length.	Minimum length.
Man	21·5	31·0	12·0
Monkey	22·4	30·0	15·0
Horse	24·1	32·0	14·0
Dog	19·9	28·0	14·0
Rabbit	19·4	27·0	14·0
Guinea-pig	24·3	31·0	15·0
Mouse	21·0	29·0	14·0
Rat	24·5	39·0	13·0

On comparing figures obtained from Table III with those from similar hosts in the case of *T. gambiense*, measured by Bruce, we get the following results:—

Table IV.

	Average length.	Maximum length.	Minimum length.
	μ.	μ.	μ.
Man—			
T. gambiense	24·3	33·0	15·0
T. rhodesiense	21·5	31·0	12·0
Monkey—			
T. gambiense	22·4	31·0	15·0
T. rhodesiense	22·4	30·0	15·0
Rat—			
T. gambiense	22·4	32·0	13·0
T. rhodesiense	24·5	39·0	13·0

This table also appears to indicate that there are some differences in size between *T. gambiense* and *T. rhodesiense.*

If now the 1000 *T. rhodesiense* are divided according to length into three groups—(*a*) short and stumpy forms of 13 to 21 microns, (*b*) intermediate forms of 22 to 24 microns, and (*c*) long and slender forms of 25 microns and upwards (as has been done by Sir David Bruce in his researches on trypanosomes), and comparison of them with Bruce's results for *T. gambiense* and *T. brucei* be made, the following percentage distributions are obtained :—

Table V.

	Short and stumpy, 13—21 μ.	Intermediate, 22—24 μ.	Long and slender, 25—39 μ.
	per cent.	per cent.	per cent.
T. gambiense	51 ·2	23 ·1	25 ·7
T. brucei	32 ·8	25 ·5	41 ·7
T. rhodesiense	36 ·1	19 ·8	44 ·1

We note that *T. rhodesiense* is richest in long and slender forms and poorest in intermediate forms.

If the percentages in the three groups are calculated for (i) each of the hosts infected with *T. gambiense* recorded in Bruce's Table III, and for (ii) each of the hosts infected with *T. rhodesiense* recorded in our Table I, then large variations are found to occur. Thus, from a comparison of 1000 *T. gambiense*, measured from seven species of animals by Bruce, on a variety of days, and 1000 *T. rhodesiense*, measured by us from eight species of animals on a variety of days, the following results are obtained :—

Table VI.

	T. gambiense.	*T. rhodesiense.*
μ.	per cent.	per cent.
13—21	32 ·0 to 82 ·1	28 ·0 to 80
22—24	14 ·3 to 33 ·3	7 ·5 to 37 ·5
25—39	3 ·6 to 52 ·0	5 ·0 to 57 ·5

Also the following table summarises the variation in 240 *T. rhodesiense* from the same rat (Table I, Rat B 16) from the 4th to the 13th day of infection.

Table VII.

	T. rhodesiense (Rat B 16).
μ.	per cent.
13—21	10 to 95
22—24	5 to 40
25—39	0 to 85

Thus it is clear that extreme variations in the length of the trypanosome are found in the different hosts, and on different days of infection in the same host, on examining the trypanosome in samples of 20.

If, again, a study of the distribution of 1000 *T. gambiense,* 1000 *T. rhodesiense,* and 1000 *T. brucei** is made by the more usual method of quartiles or octiles, the following results are obtained:—

Table VIII.

	125th.	250th.	375th.	500th.	625th.	750th.	875th.
	μ.	μ.	μ.	μ.	μ.	μ.	μ.
T. gambiense	18	19	20	21	23	25	27
T. rhodesiense	18	20	22	24	26	27	29
T. brucei	18	20	22	24	25	27	29

From this table it is seen that the measurements of *T. rhodesiense* and *T. brucei* are almost the same, but that they again differ from those of *T. gambiense.* Our results are represented graphically in Chart 1.

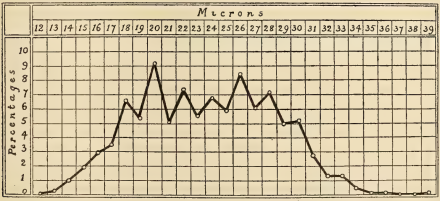

CHART 1.—Curve representing distribution, by percentages in respect to length, of 1000 specimens of *T. rhodesiense,* from various hosts.

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* The figures for *T. brucei* have been deduced as accurately as possible from Bruce's curve (1911).

We also give a chart of six hundred *T. rhodesiense* taken from the same species of host, namely, rats :—

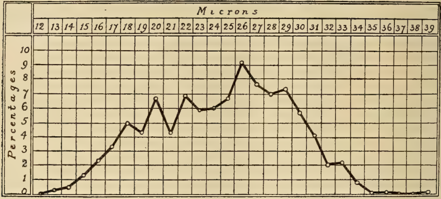


Chart 2.—Curve representing distribution, by percentages in respect to length, of 600 specimens of *T. rhodesiense* from rats. (See Addendum.)

If we now consider the graphic representation of our measurements of *T. rhodesiense*, as seen in Charts 1 and 2, and compare them with Bruce's curves of *T. gambiense* and *T. brucei*, we note the following points :—

While *T. gambiense* presents a curve with a single marked peak at 20 μ, *T. rhodesiense* presents a series of small irregular peaks extending from 18 μ to 28 μ, with the highest peaks at 20 μ and 26 μ. In the case of *T. brucei* there is a slightly irregular curve extending from 18 μ to 26 μ, with a well-marked peak at 24 μ.

Considering the three curves together, we note again that *T. rhodesiense* appears to be different from *T. gambiense*, but that the difference from *T. brucei* is slight.

Discussion of Results.

1. We consider that a sample of 20 trypanosomes, at least in the case of dimorphic species like *T. rhodesiense*, from a particular slide on a particular day is too small, as the average length obtained in this way may vary in extreme cases between 24·4 μ and 29·1 μ (see Table I, Rat B 42).

2. The day of infection on which the measurement is taken is very important, for, as we have seen in Table VII, on one day 10 per cent. of stumpy forms may be found, on another day 95 per cent. This must, we think, be due to an actual change in the number of trypanosomes of any particular length present, and not to an error of measurement.

3. It is probable also that the host from which the trypanosome is taken is an important factor. It is difficult to be quite certain of this,

because the variation may be due to the cause just stated, namely, the day of infection.

4. However, giving these sources of error due weight, we think that the fact that there is a general resemblance between the curves representing the measurements of these three trypanosomes (*T. gambiense*, *T. rhodesiense*, *T. brucei*) shows that the method is a trustworthy one.

5. The measurements of *T. rhodesiense* are much closer to those of *T. brucei* than to those of *T. gambiense*. We do not consider, however, that identity of measurement would necessarily imply identity of species. We still believe that the difference in internal morphology, namely the presence of the posterior nucleus, is sufficient to separate *T. rhodesiense* both from *T. gambiense* and *T. brucei*.

6. We think, however, that in the future, in order to get as accurate results as possible, it will be necessary on any particular day to measure larger samples than 20 trypanosomes. How large these samples must be it is, at present, impossible to say, for we have not the requisite data. This is a point we propose shortly to investigate. At present we would suggest that, in order to eliminate unknown possible variations due to the use of different hosts, samples should always be taken from the *same* animal, and, as we have shown that there are large variations on different days, samples should be taken on *every* day of the infection. Tame rats would appear to be the most suitable animals, as they are susceptible to the large majority of pathogenic trypanosomes. (See Addendum.)

Mr. Walter Stott, Honorary Statistician to the Liverpool School of Tropical Medicine, has kindly examined our figures and curves, and is of opinion that, on the whole, the data at present available are insufficient to enable statistical criticism to be applied, as there are no standard curves for comparison.

We propose therefore shortly to investigate the subject further from the various additional points of view that we have indicated.

Addendum, April 29, 1912.—Since writing the preceding we have completed a fresh series of measurements of *Trypanosoma rhodesiense* from a single rat, beginning with the first day of infection, and measuring 100 trypanosomes per day during 10 consecutive days of infection. We have thus obtained measurements of 1000 trypanosomes from the same rat. On representing the results graphically, it was found that the curve resembled that of Chart 2 (for 600 trypanosomes from rats), rising with slight irregularities to a peak at 26 μ (as does the curve of Chart 2), and then falling rapidly to 34 μ.

Our remarks on p. 232 appear to be justified, but detailed discussion must be deferred.

REFERENCES.

Bruce, Sir D. (1911). "The Morphology of *Trypanosoma gambiense* (Dutton)," 'Roy. Soc. Proc.,' B, vol. 84, pp. 327—332. 1 plate.

Stephens, J. W. W., and Fantham, H. B. (1910). "On the Peculiar Morphology of a Trypanosome from a case of Sleeping Sickness, and the Possibility of its being a New Species (*T. rhodesiense*)," 'Roy. Soc. Proc.,' B, vol. 83, pp. 28—33. 1 plate. Also 'Ann. Trop. Med. and Parasitol.,' vol. 4, pp. 343—350. 1 plate.

EXPLANATION OF PLATE 3.

Various forms of *Trypanosoma rhodesiense*, drawn at a magnification of 2000 diameters. Note that some of the short and stumpy forms have the nucleus posterior.

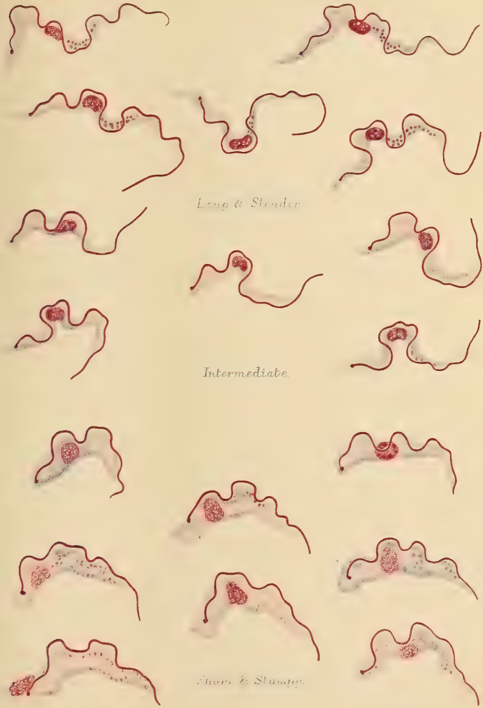
Notes on some Flagellate Infections found in Certain Hemiptera in Uganda.

By Muriel Robertson.

(Communicated by Sir J. R. Bradford, Sec. R.S. Received February 24,—Read March 28, 1912.)

While carrying out other work at Mpumu, the opportunity has presented itself of examining the Hemiptera from the surrounding country, and some interesting protozoan infections have been found. The group has a certain importance in this connection in that it includes the two parasitic genera, Cimex and Conorrhynus. Cimex has fallen under suspicion in regard to kala azar, and a species of Conorrhynus is definitely incriminated as the transmitting agent of the South American trypanosomiasis. Certain non-parasitic species, generally belonging to the group of the Reduviidæ, occasionally attack man. An instance of this has been reported by Dr. H. L. Duke from the neighbourhood of Mpumu. He has on several occasions been bitten by a hitherto unrecorded species of Henicocephalus. Cases of this kind are also known from other parts of Africa, and from India. It is, therefore, not without interest to obtain some knowledge of the Protozoa infesting Hemiptera generally, and more especially of the flagellates.

So far as I am aware none of the species dealt with here are known to attack man or other vertebrates.


Long & Slender
Intermediate.
Short & Stumpy.

M.E. Bruce, del
M.P. Parker, lith.
E. Wilson, Cambridge.

TRYPANOSOMA RHODESIENSE.

The parasites considered belong almost exclusively to the herpetomonad or crithidial type. I do not propose to attach species names, as the cycles have not been worked out, and the nomenclature is already in a very confused state. There is at present some considerable difference of opinion as to what is the correct name of the genus, and as none of the literature is accessible this question is not touched upon at all. In every case the specimens I have examined show a single broad flagellum, and I have used the term herpetomonas to describe all forms without an undulating membrane, crithidia for forms with a membrane, and with the kinetonucleus anterior to the trophonucleus. The word crithidia is not used in any generic sense but simply as a convenient term to denote flagellates showing the above-mentioned relation of membrane, kinetonucleus and trophonucleus.

Several of the insects examined have, as will be seen later, shown a confusing range of forms, whose real connection cannot be ascertained with any satisfactory degree of certainty by the mere examination of a relatively small number of natural infections. There is, therefore, every reason why species names should be withheld until these points are settled beyond doubt.

All the material was fixed by the wet method, using Schaudinn's fluid, and stained either by Heidenhain's hæmatoxylin or by Mayer's hæmalum.

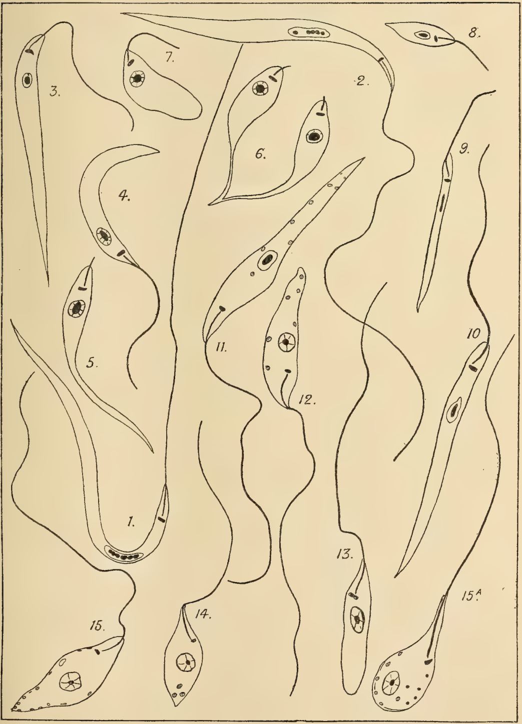
Leptoglossus membranaceus Fab. (I) is a common Uganda species; it feeds on a certain gourd-like creeper, showing a marked preference for this particular plant, and is hardly ever found except in its vicinity. The specimens are frequently infected in the gut with a long slender herpetomonas, figs. 1–7 give an idea of its general characters and appearance. The gut forms range from broad sac-like creatures with a short flagellum (fig. 7) to long slender forms with a flagellum of great length (fig. 1). The nucleus in the broad forms is of the type so common in trypanosomes and many other flagellates, and is made up of a large karyosome surrounded by a clear space, the outer limit of which is marked off by a delicate membrane; between the karyosome and the membrane there pass fine radiating strands. In the more slender forms there is to be noticed a lengthening out of the nucleus and a tendency for the karyosome to fragment (figs. 2 and 3). Finally, in the very long types, the nucleus is drawn out into a very narrow and much elongated oval, the karyosome being represented by a row of chromatin blocks of slightly irregular shapes (fig. 1). I have not been able to detect any sign of the fine strands between the karyosome and the membrane in this phase of the nucleus. The body of the long herpetomonads is markedly flexible, which is a somewhat unusual feature in parasites of this type.

Although there is no direct evidence as regards the sequence of forms in the material handled, there is little doubt, from all the work done on this type of protozoon, that the long slender creatures may be regarded as the adult individuals, and that they have been produced from the smaller broad forms. In all the infected specimens of Leptoglossus so far examined, I have not come across any of the curious crithidial forms which are so characteristic a feature of two types of infection to be discussed later.

Quite the most important point in the infection of *Leptoglossus membranaceus* is the fact that, in adult specimens, the salivary glands are very frequently strongly infected with the flagellates. Young specimens show the parasites only in the gut, and, as in the histories of most parasitic flagellates, the infection seems to work forwards, from the hinder part of the gut near the Malpighian tubules. The flagellates are not present in large numbers in the anterior part of the intestine in any of the specimens so far examined. The individuals seen in the salivary glands show a considerable range of shape and size, and curious small forms are present in fair numbers. It is not proposed to enter into these relations in detail, as this herpetomonas will, I hope, be the subject of more systematic study later on.

In the infection of the salivary glands of Leptoglossus, we have the independent development in a sucking insect of all the factors requisite for the transmission of a flagellate, parasitic in the intestine, by way of the mouth-parts of the insect host. This is a significant step in the history of how the complex interacting circumstances which we now find in operation in such diseases as trypanosomiasis, kala azar, and malaria may have arisen in nature. I have not so far found the parasite in the plant, but this part of the work has not been carried anything like far enough as yet to permit of a statement being made in regard to the point. These conditions in Leptoglossus are not at present of the slightest practical importance, but I should like to point out that it has a certain interest to find this state of affairs in Uganda involving a parasite of the same genus as that which produces the diseases of kala azar and Oriental sore.

Leptoglossus occasionally shows an infection with a coccidian, which I do not propose to describe, but there is one point that bears on the matter under discussion. This coccidian also invades the salivary glands, but probably does it by way of the body cavity. Cysts containing sporozoites were found in the salivary gland and in the gut, as also all the stages of schyzogony or asexual multiple fission. Motile, sickle-shaped individuals were found in the proboscis: morphologically, there is nothing in the live state to distinguish sporozoites and young merozoites; the probability is, however, strongly in favour of their being merozoites. I am inclined to think that the presence of these forms in the proboscis has no significance in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the coccidian cycle, but here again the condition is not without its reference to the question of the phylogenetic development of parasitic protozoa. The close relationship between the Hæmogregarinæ and the Coccidia is now well established, not to mention the general parallelism in all essential features between the cycle of the malarial organism and that of any typical coccidian. Not to lay undue weight on these points, the main feature here, as in the previous instance, is the arrival of an intestinal parasite in the mouth-parts of a sucking insect, which is, however, still a plant sucker.

The concurrence of the flagellate and coccidian parasites in the Leptoglossus and the fact that they have reached the salivary gland in both cases is rather striking in view of the frequent association of flagellates and Hæmogregarines in the blood stream of vertebrates.

Dysdercus casiatus (XIV), the red cotton-bug, shows an occasional infection with a typical herpetomonas; so far the parasites have only been found in the gut. Only a small number of infected specimens have been examined, figs. 8–10 show the general characters of the parasites; no crithidial forms have been found in these infections.

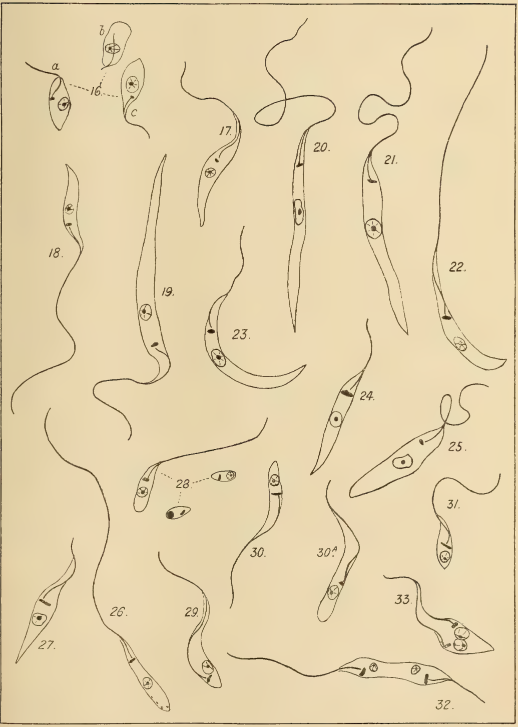
Carbula jipensis (X).—This small bug shows rare and generally slight infections with a herpetomonas.

Two sets of infection (one in a reduviid XV, the other in a capsid bug V) now come to be considered which differ from those hitherto handled in that crithidial forms are present in large numbers as well as the typical " needle-and-thread " herpetomonads and the broad forms from which they spring. It is almost impossible to come to a really satisfactory decision as to whether there are two different species present or whether all the forms represent different stages of one species. I am inclined to consider, however, that in each of the two cases one is dealing with a single parasite, and that the crithidial forms, though sometimes present in large numbers, are more or less transitory growth forms, and that the slender herpetomonads (11, 20, and 21) are the adult individuals. Nevertheless the opposite view could be so well defended and has so much in its favour that it must be considered as practically an open question until more evidence is to hand.

The facts that, in my opinion, weigh the scale in favour of one species in which the long herpetomonads are the adults and the crithidia transitory growth forms are as follows, and apply equally to either of the two sets of infections :—

1. The herpetomonad infections are sometimes found unaccompanied by the crithidial forms, but the converse has never been observed.

2. The crithidial forms are generally present in strong, actively multiplying infections.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3. The crithidial forms merge into the herpetomonad types.

The appearance of the crithidial forms is probably due to the rapid growth of the flagellum, which is finally very long, causing the displacement of the kinetonucleus. The flagellum is in any case attached to the periplast for part of the way, and the development of the membrane in these forms is never more than a very narrow ridge of periplast along which the flagellum runs It looks as though when the body lengthened out this uneven growth were compensated and the herpetomonad type was reached, partly by the drawing out of the posterior end of the body, and partly by the moving forward again of the kinetonucleus.

Many herpetomonad infections show this confusion of types, for instance that of the house-fly in Europe and also in India. No work has been done on these forms which has clearly shown the connection of these various forms one with another. In the house-fly the difficulties in the way are very great, and the life-histories of herpetomonads seem to be in need of further study. It is hoped that the form in Leptoglossus may be worked at in greater detail, as this bug breeds readily in captivity and is easily fed.

The first of the infections just discussed occurs in a bug belonging to the family of the Capsidæ (V); it is found in or near forest and is of the same light brown colour as the fallen leaves on which it runs. Its food is not known and it is apparently not a very common bug. Figs. 11–19 illustrate the main types found in this infection.

The second of these infections was found in a red and black reduviid (XV) which has not as yet been identified. It is a common species and feeds on small bugs and on other insects; the specimens are frequently infected, but I have not so far found any showing the flagellates in the salivary glands. The herpetomonas is small in size, and some of the infections, notably those in which the crithidial forms are numerous, show large numbers of small rounded forms without a flagellum. Figs. 20–33 show the features of the parasite more clearly than any mere verbal description.

I am indebted to Mr. Gowdey, the Government entomologist, for kindly identifying the species named.

Notes on Certain Aspects of the Development of Trypanosoma gambiense *in* Glossina palpalis.

By Muriel Robertson.

(Communicated by Sir J. R. Bradford, Sec. R.S. Received February 26,—Read March 28, 1912.)

In the course of an attempt to obtain an insight into the details of the life-cycle of *Trypanosoma gambiense* in *Glossina palpalis*, certain experiments were undertaken involving the feeding of a relatively large number of flies under closely observed conditions. Although primarily undertaken with a view to the morphology and development of the parasite, they have a bearing on the general relation between the trypanosomes and the Glossina that is of some interest.

The present account deals with the infections produced in the flies as a whole; the morphological results will be considered in detail elsewhere. I must point out that the experiments in question are not concerned with actual transmissions of *T. gambiense* from an infected to a clean host, but with the number of flies in which the trypanosomes will develop. That flies harbouring trypanosomes are infective from about the 26th day onwards has been shown over and over again; it was therefore considered to be a wanton waste of life to allow every cage kept beyond the 30th day to infect a clean animal. Late cages were usually fed on cock's blood after the 24th day. A small proportion were actually tested and an infection was invariably produced if the box contained flies showing trypanosomes.

There is no evidence to show that a trypanosome-infection once established in the fly is ever got rid of subsequently. *T. gambiense* may be held to be established if the gut shows trypanosomes after the 5th day in flies which have had *at least one feed of clean blood* subsequent to the infecting feed.

This last statement bears on a point of some importance; it has been found during the course of these experiments that flies allowed to have one infected feed and then starved absolutely, when dissected between the 6th and the 12th day, show an extraordinary number of individuals in which trypanosomes are to be found. Flies starved in this way rarely live beyond the 12th or 13th day. These experiments will be referred to as starvation experiments.

Of 103 flies so treated and fed (for one infecting feed only) in groups on different monkeys infected with *T. gambiense*, 22 showed trypanosomes between the 6th and 12th day, that is to say 21·3 per cent. of the flies harboured trypanosomes. Six flies of the total 22 showed trypanosomes only in the sucking stomach, or crop, as this organ should perhaps be more appropriately called, 13 showed a well-established infection in the gut and three showed trypanosomes in both situations. It is perhaps advisable to neglect the six flies in which the parasite was only present in the crop, although a certain amount of development may go on in this organ; the percentage thus obtained is still very high, namely 15·5.

Monkeys infected with *T. gambiense*, and probably most other animals with trypanosomes in their blood, have negative periods, that is to say, periods during which they do not infect flies. A number of the experiments have shown that trypanosomes may be found by microscopic examination, although the blood is not infective to flies. It is interesting to note that such negative periods appear to be negative in starved as well as in fed flies. This relation is, however, not yet sufficiently worked out. In the starvation experiments the microscopic appearances do not, so far as I have yet seen, show any distinction from those to be observed in established infections in fed flies of the corresponding ages.

The crucial moment in the cycle appears to be the first feed of clean blood subsequent to the infecting feed. It is not evident if this clearing out of the trypanosomes by the clean feed is a purely mechanical action due to the flooding of the gut or is a result of the general change of condition thus brought about.

In any case the number of flies containing trypanosomes obtained in starvation experiments during periods when the vertebrate is in the infective condition would give the maximum register of the potential infectivity of that individual strain to fly. The actual number of flies containing trypanosomes from parallel experiments, which were, however, subsequently fed, would give an indication of the additional inhibiting power of the fly under ordinary conditions whatever the cause to which the inhibition may be due. Experiments of this type were undertaken but gave no result, as the whole series proved negative, the monkey not being in the infective condition.

The total number of flies used in the whole group of experiments under consideration in this paper is 1411 males and 1322 females, of which 42 males and 39 females show trypanosomes. Irrespective of sex, the total number is 2733, of which 81 gave a positive result.

From this total must be deducted the starvation experiments and a small group which are not strictly comparable, owing to the feeding having included toad's blood, and also those flies dissected before the 5th day. Concisely the figures stand thus:—

Total	2733 flies;	infected flies, 81.
Toad and starvation experiments......	234 „ ;	„ 17.
Flies dissected between days 1—5 ...	84 „ ;	„ 9.

Deducting the last two batches from the total there remain 2415 flies, of which 55 were infected; that is 2·27 per cent. of the flies harboured trypanosomes.

This percentage, *i.e.* 2·27, is naturally not the measure of the infectivity to the fly of any strain (or strains) of trypanosome. It is the percentage of infected individuals produced by allowing 2415 flies to feed at random, in groups, through a period of two and a half months, on a population of nine infected monkeys. Each group receives of course only one or two feeds on the infecting monkey, and is then fed on clean animals.

Certain obscuring features, habitually neglected in dealing with trypanosome infections, must be pointed out in figures handled in this way. The nature of the individual strain must be considered, and the occurrence of negative periods (*i.e.* periods when the vertebrate is not infective to fly) must be duly taken into account. As they stand, the figures above cited have no real meaning. The number of infected individuals obtained by feeding flies at random upon an infected monkey or other vertebrate is neither an index of the infectivity of the strain nor of the potential danger of such an animal at large in a fly area. The percentage, however, of infected individuals produced among flies fed during periods when the blood is infective, gives the index of the virulence of the strain as regards fly. If, on the other hand, batches of, say, 50 or 100 flies were fed on an infected monkey for every day of its life during the course of the disease, the infected glossinæ thus produced would give an index of the infective power of the monkey as a whole.

It is obvious that there are two quite different aspects of the question, and calculations in which they are treated as one must naturally be misleading. In practice it seems usual to neglect this distinction, with the result that there has been a tendency to underestimate the potential transmitting capacity of the fly, and to over-rate its individual idiosyncrasy. Given reasonably favourable conditions of temperature and moisture, it is the strain of trypanosomes and not the fly that within a relatively wide range plays the deciding *rôle* in limiting the number of infected glossina. There is, of course, as has already been mentioned, a serious difficulty in the way of the trypanosome in its attempt to establish itself at all in the glossina, but that must be very nearly constant in all cases.

To consider some of the experiments in greater detail. Monkey 113, infected by wild flies from the lake-shore, first showed trypanosomes in its blood on July 25, 1911. On August 23, Monkey 113 showed trypanosomes in its blood; 137 flies were fed in groups, 36 of these were treated as a starvation experiment; the whole series proved negative with the exception of one starved cage, which showed one infected fly on the 12th day. On August 24, 45 flies were fed on the same monkey, and there resulted five infected flies, that is, a percentage of 11·1 showed trypanosomes. On August 25, 53 flies were fed, of which two were infected, which is equal to a percentage of 3·7.

Table I.—Monkey 113.

Date.	Condition of blood examined alive.	+ or − laboratory flies.	Number of flies fed.	Percentage of + flies.	Number of experiment.
Aug. 20	No trypanosomes seen in blood ...	No flies fed	—	—	—
„ 21	„ „ ...	„	—	—	—
„ 22	„ „ ...	„	—	—	—
„ 23	Few „ „ ...	—	101	—	38—42
„ 24	Numerous trypanosomes seen in blood	+	45	11·1	44 and 45
„ 25	„ „	+	53	3·7	46
„ 26	Very numerous trypanosomes in blood	—	89	—	47—49
„ 27	Very few trypanosomes seen in blood	No flies fed	—	—	—
„ 28	Showed no trypanosomes in blood	— (flies fed both days)	25	—	50
„ 29					
„ 30	Shows few trypanosomes	—	34	—	55 and 56
„ 31	„ „	+ (box fed on 30th and 31st)	107	1·8	54
Sept. 1	Not examined.				
„ 2					
„ 3					
„ 4	Very few trypanosomes	—	32	—	58
„ 5	„ „	No flies fed	—	—	—
„ 6	Fair number of trypanosomes; dimorphism of broad and narrow forms very marked	—	49	—	60
„ 7	Fair number of trypanosomes ...	No flies fed	—	—	—
„ 8	Trypanosomes very numerous indeed	+	95	2·1	62 and 64
„ 9	Fair number of trypanosomes, but much fewer than on 8th	No flies fed	—	—	—
„ 10	Not examined.				
„ 11	Few trypanosomes	No flies fed	—	—	—
„ 12	Trypanosomes very numerous indeed	+	70	1·4	68
„ 13	„ „	No flies fed	—	—	—
„ 14	„ „	„	—	—	—
„ 15	Blood swarming with trypanosomes	+	20	10·0	75

On August 26, however, 89 flies were fed in three groups on this same monkey, and produced no infected flies at all. The experiments ran concurrently, and shared the same weather and other external conditions, and were similarly fed. Here, as all through this paper, results of flies dissected before and on the 5th day are excluded. In the experiments just mentioned the flies were dissected at different periods, but all after the 20th day. One is forced to the conclusion that, although the monkey showed trypanosomes on all the four days in question at the time of feeding the flies, the blood was only very slightly infective on the 23rd, probably not at all to fed flies; that on the 24th and 25th it was infective, producing (adding the results for the two days) seven infected individuals out of 98 flies, *i.e.*, 7·1 per cent.; and that it was once more non-infective on the 26th. Under conditions so similar it is impossible to consider a difference of 7·1 per cent. to be due to the individual variations of the two laboratory-hatched sets of flies. The onus of this discrepancy must obviously be borne by the trypanosomes derived from the monkey.

It is interesting to note in this connection the result of microscopic examination of the live blood of this monkey over these and the adjacent days.

For the sake of completeness I add the percentage of infected flies produced over all the boxes containing infected flies obtained from Monkey 113. This includes some results from dates earlier than those of the foregoing table. Starvation results and flies dissected before the fifth day are as usual excluded.

	Flies.	Infected.
Experiment 25	69	2
,, 29	60	1
,, 32	71	1
,, 43	32	3
,, 45	13	2
,, 46	53	2
,, 54	107	2
,, 64	104	2
,, 68	70	1
,, 75	20	2
Total	599	18

The percentage of infected flies is equal to 3 per cent. I do not add the percentage obtained by including all the boxes in which the result was negative. The number so obtained would be a mere numerical curiosity, as, with our present knowledge, it is purely a matter of chance from the point of view of the experimenter, how often he happens to strike a non-infective period. It is obvious that a quite uncontrollable factor is introduced if the figures are so handled. It becomes clear that although a trypanosome-infection is in a continual state of flux the percentage of plus flies produced over infective periods gives a measure of the virulence of the strain to fly and forms a basis of comparison between different strains.

Another set of experiments bears on this point. Monkey 199 was infected by Dr. Duke by direct injection of blood from a bush-buck which had been infected with *T. gambiense* by laboratory infected flies. The bush-buck had harboured *T. gambiense* for 15 months. This monkey showed infective and non-infective periods in exactly the same way as other infections, but the infective periods gave quite an unusual number of flies harbouring trypanosomes. Thus—

Experiment 71, 13/9/11, 54 flies fed, gave 4 positive, = 7·4 per cent.
„ 70, 14/9/11, 50 „ „ 7 „ = 14·0 „
„ 74, 15/9/11, 46 „ „ 5 „ = 10·8 „

Considering all the figures together, out of 150 flies, 16 showed trypanosomes, that is a percentage of 10·6 per cent.

This relatively very high percentage was also borne out in experiments of Dr. Duke's in which he made use of this monkey and which he kindly permits me to quote. Thus of 188 flies from two experiments the conditions of which admit of comparison with those of Experiments 71, 70, and 74 just cited, 11 were infected, that is 5·8 per cent.

Taking this set of figures with those quoted above, of 338 flies, 27 were infected, which is equal to a percentage of 8 per cent. This is more than double the normal percentage of infected flies produced by the Uganda strain of *T. gambiense* in monkeys. All the conditions being considered, it is impossible to attribute this difference to anything but the strain of trypanosomes.

Besides having a virulent character as regards the production of infected flies as a whole, an individual strain has often a recognisable type or method of development in the glossina. For instance, all the flies fed on Monkey 199 gave very numerous and rapidly developing infections; the trypanosomes reached the proventriculus earlier in the cycle than is usual, and were established in the salivary glands much more promptly than in the case of ordinary cycles. One cage was infective on the 24th day. This difference of character appeared in the flies from Dr. Duke's experiments, as well as in those cited above, and I am indebted to him for the opportunity of examining them. The monkeys infected by fly fed on Monkey 199 showed good infections in the blood (Monkeys 330, 390, and 391), but flies fed on these monkeys gave only an average number of infected flies, *i.e.* 3 per cent., which, however, showed rather sluggish and very slowly developing infectious. Thus, one of the cages fed on one of these monkeys showed an infected fly in which the infection had not yet reached forward beyond the mid-gut on the 22nd day, and another on the 56th day, in which the salivary glands were not yet infected. There was no possible chance of a "pick up" infection in either case. A stray fly, showing a very backward infection, is generally due to having allowed a cage to stay too long on the test animal, that is until after it has produced an infection. A new cycle may then be started in a fly which had escaped on the previous occasion. This point is, I may mention in passing, another argument in favour of the failure of the trypanosomes to establish themselves in the fly being due rather to the flagellates than to any absolute inhibiting quality or condition in the recalcitrant glossina.

Monkey 199 illustrates some particularly important points in regard to the cycle of *T. gambiense* as a whole. It has been shown, by many experiments carried out at Mpumu, that infected buck produce a high percentage of plus flies, but that monkeys infected by means of these flies give in turn only the usual low percentage characteristic of cycles started from monkeys.

The important features are—

1. The long period during which the trypanosomes had been in the buck, namely, 15 months.

2. The infection of 199 by direct injection of the blood from the bush-buck.

3. The large percentage of infective flies yielded by 199 when infective.

4. The loss of this last character when the strain is transmitted by flies to other clean monkeys.

Now, the result of a great deal of biological work during recent years has been to establish the general idea that the function of conjugation or nuclear fusion is not reproduction, but the preservation of the characters of the species as a whole, and the neutralisation of the undue tendency to variation produced by unchecked individual multiplication. In the case of trypanosomes, the individuals run through a relatively very large number of generations in the vertebrate, and in consequence, as is well known, are capable of developing very well marked strains, which might almost be termed varieties. The function of the fly, as is obvious from these experiments, is to sift out these variations of the individual strains, and to produce a fairly even type. There is at present no sound evidence of conjugation in any trypanosome life-cycle so far worked out, and the question must be left unprejudiced. It is a very plausible suggestion that the great and undoubtedly stimulating change of environment that occurs in the alternation of hosts has gradually led to the suppression, and finally taken the place of conjugation. This hypothesis would explain the labile characters and the extraordinary merging of species in the trypanosome group, but it is obviously open to much criticism on the score of its speculative nature. Further, such a conception stands only so long as no sound evidence of conjugation in any trypanosome cycle is to hand.

In any case it seems clear that the cycle in the fly as a whole, whether conjugation actually occurs or not, has much of the biological significance of that process. This conception is of some importance to workers dealing with laboratory strains passed directly for long periods without reference to the intermediate host.

It might be suggested that the fluctuation in the infectivity of the blood of the vertebrate, on which these experiments have laid particular stress, is due to the coming and going of sexual forms in the peripheral blood. The microscopic appearances in the vertebrate have not as yet been sufficiently closely correlated with the infected periods to permit of any statement as to whether there is a morphological distinction in the individuals at different times.

This paper does not deal with the morphological aspect, but I should like to deprecate the rash use of the terms male and female to describe slender and broad trypanosomes. In *T. gambiense* cycles, in the glossina, very slender elongated forms are produced from the broad forms, as in most other trypanosome life-histories; they are naturally present together in the posterior part of the gut, but the former are destined to pass forwards and form the overwhelming majority of the individuals in the proventriculus and thoracic intestine. The terms male and female are gratuitously misleading until the individuals have been shown to have a sexual connection.

Such a use of language encourages an undue bias in the mind of the observer, and, while I am far from denying the possible and even probable occurrence of a sexual process, it is obviously an unscientific procedure to select the individuals *a priori* by the casual method of analogy, an analogy, moreover, not even drawn from the same class of the protozoa.

Petrifactions of the Earliest European Angiosperms.

By Marie C. Stopes, D.Sc., Ph.D., F.L.S., Fellow of University College, London.

(Communicated by D. H. Scott, F.R.S., Pres. L.S. Received February 20,—Read May 2, 1912.)

(Abstract.)

The paper records the existence of Angiosperms in England in Aptian times, *i.e.* at a geological period when they have been hitherto supposed not to exist in Northern Europe; describes botanically the anatomy of these fossils, which come under three new genera; and notes the points of structural and phylogenetic interest in them, as being the oldest Angiosperms of which the anatomy is preserved, and contemporaneous with Bennettites.

The specimens are from the Lower Greensand; all are in the Geological Department of the British Museum. All are woody Angiosperm with secondary thickening, of which the internal anatomy is preserved. In one of the specimens the petrifaction of the tissues is remarkably beautiful, showing the pit canals in the wood fibres, ray cells, etc.

The three specimens differ so much that they are put into three different genera to which entirely non-committal names are given, as there is no evidence that they belong to any extant family. The plants described are shortly:—

Aptiana radiata, gen. et spec. nov., a woody stem about 3·5 cm. in diameter, with pith and cortex preserved. The vessels are exceedingly small, comparatively evenly scattered, little disturbing the rows of fibre-tracheids composing the uniform wood. The rays are numerous, uniseriate, and three- to four-seriate, the latter with funnel-shaped expansions in the phloem.

Woburnia porosa, gen. et spec. nov., part of the secondary wood, with large numbers of exceedingly large vessels, and broad rays.

Sabulia Scottii, gen. et spec. nov., decorticated woody stem with pith, in which all the tissues are much thickened, and the vessels principally in pairs.

All are undoubted Angiosperms, which cannot be referred to any living form, though in individual details of structure they resemble one or other of the families of modern Angiosperms. That they are contemporaneous with Bennettites gives them a further point of theoretic interest. Their chief importance lies in the fact that they are the first Angiosperms to be described from this early horizon in Northern Europe, and that they are the oldest Angiosperms of which we know any part of the anatomy.

*On the Instability of a Cortical Point.**

By T. Graham Brown and C. S. Sherrington, F.R.S.

(Received March 16,—Read May 2, 1912.)

(From the Physiology Laboratory of the University of Liverpool.)

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I. Introduction.

Slight and transient differences in the depth of narcosis, in the freedom of blood supply, and in the temperature of the preparation modify reactions obtained from stimulation of the motor area in the cortex cerebri with a facility and to a degree not met in the study of purely spinal or decerebrate reflexes.

The observer, when first attempting to decipher the rules exhibited by the reflex activities of a spinal or of a decerebrate preparation, naturally turns first to those results which lend themselves best, by regularity and harmony, to exact formulation. Occasional exceptions as they arise are reserved for examination in the future. In a similar manner, the codification of those responses which are obtained with the greatest reliability from the several cortical points is what at first chiefly concerns the observer in the study of the elicitation of different movements from different points in the cortex cerebri.

In the present observations, however, our aim has been rather to note to

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* The expenses of this research have been in part defrayed by a grant from the Carnegie Trust.

what degree the reaction obtained from one and the same topographical point of the cortex may vary from time to time.

In doing so, we are following a trend of enquiry very noticeable in recent experimental study of reflex and other nervous reactions. The reversal (" Umkehr ") of the sense of the movement elicited by identical stimulations of the same reflex preparation as determined by this or that accessory change in the other conditions of the experiment has been a frequent theme in recent papers. Such observations, besides tending to throw light on the reflex working of the neural machinery, have the further interest that they tend—although perhaps remotely—to make some approach towards study of the physiological conditions which underlie " will," if so bold a use of the term may be allowed.

Variations of the muscular response given by one and the same point of the motor cortex conformably with variations in the degree of narcosis, etc., have been met often in our observations. Such have frequently been described by other experimenters; and, since they are well known, we do not intend to discuss them now. We wish, however, to call attention to some other sources of variability of the cortical responses, instances of alteration and of reversal more closely analogous to those recently studied in the case of reflex preparations.

II. Methods Employed.

The material used in these present observations has been provided entirely by the monkeys and the anthropoid apes. One chimpanzee (*Troglodytes niger*) has been used, one baboon (*Papio anubis*), and the remainder ordinary small monkeys (*Macacus rhesus, Macacus sinicus, Callothrix*). All of the experiments were done under chloroform and ether narcosis.

For the purposes of graphic registration (as in the majority of the observations), two muscles which antagonise each other at the elbow—*supinator longus* and the humeral head of the *triceps*—have been used. These have been isolated, and the complication of movement of other than the selected parts has been abolished—by the motor paralysis of the other muscles at the shoulder, elbow, and wrist. The muscles so treated have been—trapezius, deltoid, supraspinatus, infraspinatus, teres major, teres minor, latissimus dorsi, scapular head of triceps, pectoralis major, pectoralis minor, biceps, and coraco-brachialis, and all the muscles of the fore-arm with the exception of the supinator longus. The lower end of triceps has been freed by division of the olecranon process and connected directly by a thread with one of the recording levers. The supinator longus has not, however, been interfered with, but the elbow has been allowed to swing free, and the movement of that muscle recorded by registering the movements of the lower end of the fore-arm.

To fix the origins of the recording muscles, the humerus was transfixed by steel holders at the head and through the condyles. This fixation was of sufficient firmness to prevent the movements of other muscles from complicating the records.

Cortical stimulation was applied by the unipolar method. Two electrodes were so arranged that either could be used separately at will. The secondary circuit was completed by the simultaneous closure of two keys, one of which was in the wire of the unipolar electrode, and the other of which was in the path of the indifferent one. A third key was closed mechanically at the same time. This made a signal circuit. A similar arrangement of keys was used in the case of the second electrode. The advantage of this arrangement, when both electrodes were on the cortex, was that there was no danger of a short circuit through one electrode, as might be the case if a system of short-circuit keys was used.

The stimulating arrangements were such that the circuits might also be applied either to the contralateral or to the ipsilateral ulnar nerve by the bi-polar method, so that peripheral stimulation might be applied at will.

The left arm muscles were used for registration in all the observations. In the following descriptions, the words contralateral and ipsilateral always refer to the locus of the stimulus in reference to the observed arm.

In regard to stimulation of the cortex, our general procedure has been to seek out in the arm area two points, one yielding primary flexion at the elbow, and the other primary extension. In our experience a flexion-point can usually be found somewhat more easily than can be an extension-point; and the field of cortex from which the former can be selected is the wider. Points yielding extension lie for the most part higher up upon the convolution (*gyrus precentralis*) and nearer to the dorsal limit of the arm area. But the points which best yielded extension have varied unmistakably in their exact position from experiment to experiment—although sometimes closely corresponding in situation. In some experiments, the area whence extension-points could be chosen has been distinctly larger than in others. Our impression from the observations has been that this variability signifies less a difference in the permanent arrangement than a difference in the condition of the nervous system from time to time. In one case the same point which yielded primary extension with much regularity, on re-examination 28 hours later in the same animal, yielded at first primary flexion instead of the usual primary extension. The cause of this difference between experiments carried out under regularly similar conditions we are unable to assign.

By an extension response we mean one in which the primary movement is extension as regards the isolated muscles under observation at the elbow. By a flexion response we mean conversely one in which the primary movement in these muscles is flexion. It may be added that a flexion response has in our preparation two modes of expressing itself; it can either cause contraction of the flexor muscle, or it can cause inhibitory relaxation of the extensor muscle—but this latter form will be obvious only if the extensor be in some degree of contraction at the time when the inhibition occurs. Very often the two effects—contraction of flexor and inhibitory relaxation of extensor—result together (reciprocal innervation). But sometimes only the one or the other is observable, and of these the evident one is frequently the inhibitory relaxation. Similarly, an extension response has two means of expressing itself in the preparation—namely, either in contraction of the extensor muscle or in inhibitory relaxation of the flexor muscle—and very often it expresses itself in both of these ways together, but sometimes by one or the other alone.

For brevity, a cortical point which, under ordinary conditions of stimulation, regularly yields a primary flexion response may be termed an "F" point; and similarly a cortical point which regularly yields a primary extension response may be termed an "E" point.

III. Changes in the Response of a Cortical Point in Result of Serial Stimulation of it.

We find that successive stimulations of one and the same cortical point at short intervals of time usually produce, as is well known, marked facilitation and intensification of the response. This is clearly demonstrated when stimulations of two seconds' duration are repeated at two seconds' intervals without change in their intensity or other characters (figs. 2, 3, 4, 5). The responses in the earlier portion of the series tend to exhibit a regularly progressive shortening of the latent period (figs. 4, 5) and an increase in the amplitude and intensity of the muscular effects, and this increase in some cases proceeds through a long series of responses, so that although the opening member of the series is very weak, the last may produce a maximal contraction of the muscle, or, if that is already present, may maintain it (fig. 4).

Sometimes, however, the character of the response exhibits changes qualitative as well as quantitative. Thus a point which began by yielding primary extension may come to yield primary flexion in the latter part of the series. Its action undergoes reversal. Such reversal, although taking place *gradatim*, may in some cases develop very rapidly (fig. 1).

Similarly an F point yielding primary flexion at the beginning of a series sometimes changes its response so as to yield primary extension even after a comparatively small number of repetitions.

Reversals of this kind tend in our experience to occur more frequently with E points than with F points. The reversal may evidence itself as relaxation of one muscle along with contraction of the other, or it may be

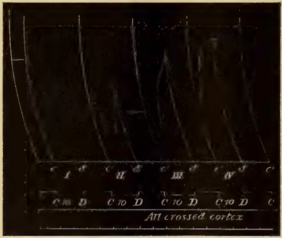

Fig. 1.

Experiment 13, *Callothrix*, 19.2.12.—Record of a series of four successive stimulations of an extensor ("E") point, each stimulation being of a duration of about 2 secs., with intervals of 2 secs. between successive stimuli. In this record the movements of the *flexor* muscle alone are reproduced. At the first application of the stimulus the flexor relaxes. In the succeeding interval there is a partial recovery of the flexor tonus. The second stimulus evokes relaxation, followed by contraction, through the period of stimulation. The third gives a shorter phase of relaxation and a greater contraction. At the fourth application of the stimulus there is a response of almost pure contraction.

In this and in all succeeding records the rise of the curve denotes contraction of the recorded muscle, and the fall relaxation. Below the muscle tracings are two signal lines. In these the fall of the signal denotes the beginning of stimulation and the rise the end of stimulation. These points are marked by capital letters (C, D, E, F, A, B, X, Y), and on the muscle tracings ordinates which correspond in time to the signal marks have been drawn. The ordinates bear the corresponding small letters. In the signal lines the numerals give the relative strengths of stimulus as measured by the distance in centimetres between the primary and secondary coils. The lowest line marks time in seconds.

In all succeeding figures the movements of both of the antagonistic muscles are reproduced. The upper tracing is that obtained from the *flexor*—supinator longus; and the lower tracing is that obtained from the *extensor*—the humeral head of the triceps. A millimetre scale has been drawn upon the tracing before it was varnished, and is therefore reduced in the same ratio as the remainder of the record. In many other records there are additional ordinates drawn upon the two muscle tracings, to demonstrate the correspondence in time of different parts. These are numbered in Arabic numerals. The letters "CP," sometimes present in the signal lines, mean that in these records the primary current was broken immediately after the cutting of the secondary circuits.

limited in appearance to relaxation alone—that is, to the replacement of contraction by inhibitory relaxation.

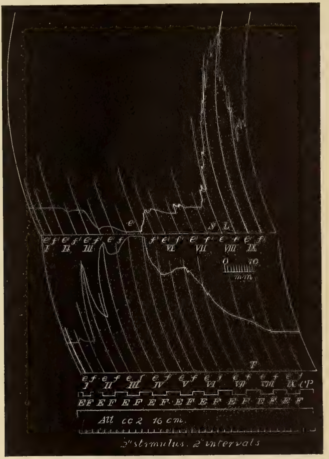

Fig. 2.

Experiment 14, *Macacus sinicus*, 21.2.12.—Record of a series of nine successive stimuli of the same strength applied to an extensor-point. The first four of these evoke flexor relaxation and extensor contraction, but the fifth gives a reaction of flexor contraction and extensor relaxation, and this reversed reaction continues in the succeeding stimuli. This shews reversal of an extension reaction to one of flexion during repetition of the same stimulus applied to the same cortical point. It will be observed that at first there is built up an extensor tonus, and that in the terminal part of the record a flexor tonus is produced. It may further be seen that with the production of these states of tonus there is a reciprocal diminution of the tonus of the antagonist.

Amongst these reversals a case which should perhaps be considered apart as belonging to a special category is that in which a flexion-point when restimulated during the course of an epileptoid discharge started by its own precedent stimulation produces, as it not infrequently does, prompt inhibitory relaxation of the flexor muscle which it previously threw into

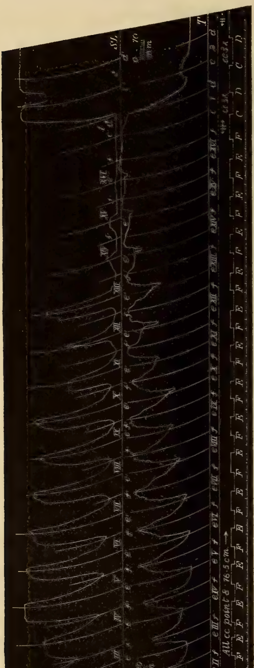

Fig. 3, *a*.

Experiment 15, *Callothrix*, 4.3.12.—The two portions of this figure are directly continuous.

In the first portion (*a*) there is at first a series of sixteen successive stimulations of the same extension-point and with the same strength of stimulus. These are numbered with Roman numerals. This demonstrates a successive facilitation of the extensor reaction, the extensor contractions increasing in extent along with a reciprocal increase in the extent of the flexor relaxations. There is also a well marked production of extensor tonus, and it will be noticed that the extensor contractions do not summate upon this. There is almost no evidence of reversal by repetition of stimulation here, although the preliminary relaxations of the extensor, which appear for the first time at stimulus "X," may be evidence of such a reversal. In this part of the record the two terminal stimuli (Arabic numerals, 1, 2) are those of a flexion-point. The first gives some extensor relaxation and a strong flexor rebound. The second gives a partial flexor relaxation, followed during stimulation by a flexor contraction. The tracing ends in a state of marked flexor tonus, which is seen to be more rapidly established than is the extensor tonus.

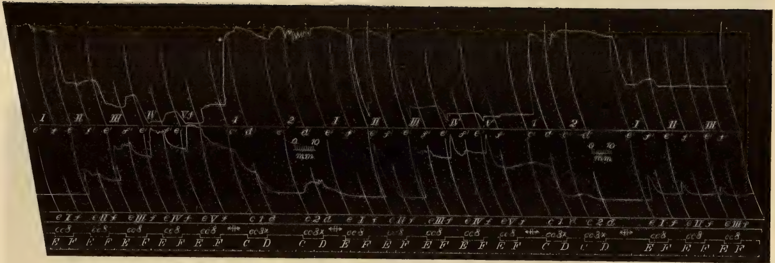

Fig. 3, *b*.

In the second portion of the tracing there is, first, a series of five stimuli of the same extension-point as before. At the first of these there is a slight augmentation of the state of flexor maintained contraction and no extensor contraction. In the second there is, however, extensor contraction and a great flexor relaxation. The series then proceeds as before—the extensor tonus rising with a reciprocal fall of flexor tonus. Stimulation of the flexion-point is again twice repeated (C—D, Arabic numerals 1, 2). A state of maintained flexor contraction is at once established. At the second stimulus both muscles are seen to contract. There is again repeated a series of five stimuli of the same extension-point as before. The first of these gives no obvious reaction. At the second there is a good extensor contraction and flexor relaxation. There is then a restitution of the flexor tonus, which is, however, finally broken down at the next stimulus, at which a state of maintained extensor tonus is again established. This is done more rapidly than before. After this two flexion-point stimuli re-establish the state of maintained flexor contraction, and this is again broken down by the repetition of the extension-point stimuli, even although there is not then produced a state of maintained extensor contraction.

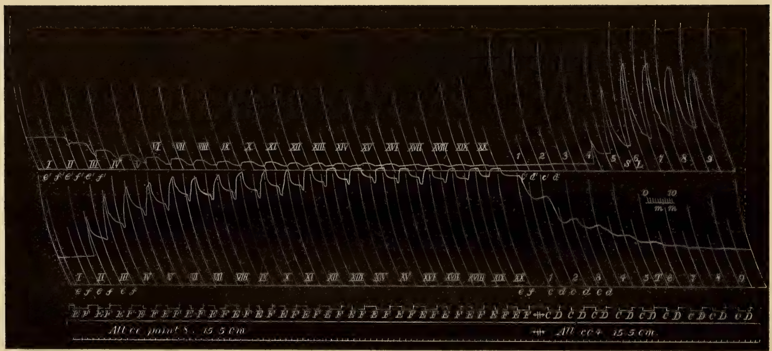

Fig. 4.

Experiment 15, *Callothrix*, 5.3.12.—Record of a series of twenty successive stimulations of an extension-point, followed by a series of nine successive stimulations of a flexion-point. The first series demonstrates very clearly the facilitation of the extension reaction by repetition of stimulation. It also shows the establishment of a state of maintained extensor contraction and of reciprocal flexor relaxation. There is a slight evidence of reversal by repetition of stimulation in the preliminary extensor relaxation which first appears in stimulus IV. The flexion series is of interest in that it shows that in this case the first appearance of the reaction was as extensor relaxation, and that it was only in the fourth repetition of the stimulus that the flexor contraction first appeared. Here the state of maintained flexor contraction is present, but is not so marked as in the previous figures.

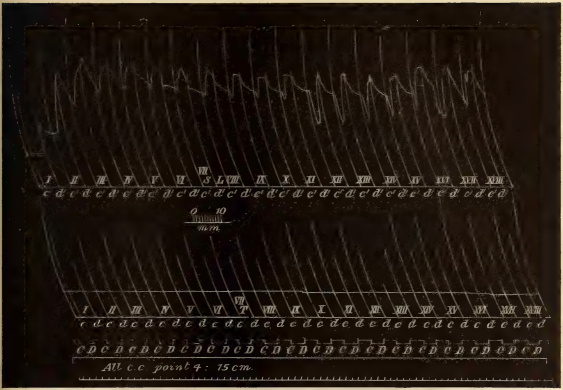

FIG. 5.

Experiment 15, *Callothrix*, 5.2.12.—Record of a series of seventeen successive stimuli applied at the same strength to the same flexion-point of the cortex. This demonstrates at first facilitation of the reaction and an establishment of a state of maintained flexor contraction. After stimulus X the reaction becomes diphasic, there being at first relaxation followed by contraction. This, which is particularly well marked in the case of stimulus XII, shows a partial flexion reversal by repetition of stimulation.

contraction (fig. 6). This reversal, however, does not always take place; sometimes the restimulation of the point tends to increase the contraction of the muscle although it does at the same time lessen the clonic factor in the epileptoid discharge while augmenting the non-clonic factor.

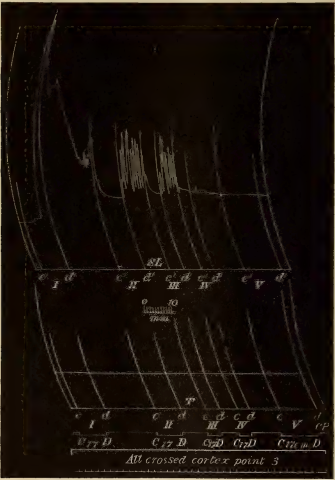

Fig. 6.

Experiment 14, *Macacus sinicus*, 21.2.12.—Record of a series of five successive stimuli applied to a flexion-point on the cortex. The first evokes a flexor contraction, which is followed by slight epilepsy. The second stimulus is applied during this, and instead of giving a flexor contraction, produces a relaxation. After this the epilepsy becomes more evident, and a third stimulus again reduces it. The epilepsy is again established at the cessation of the third stimulus, and is again reduced by the application of the fourth stimulus. The flexor epilepsy is not again established, and a fifth stimulus gives no apparent reaction. This record demonstrates reversal during epilepsy.

IV. Change in the Response of a Cortical Point Subsequent to Stimulation of a Cortical Point Antagonistic to it.

(a) *Change in the Response of an Extension-Point subsequent to Stimulation of a Flexion-Point.*

The relative constancy of the characters of the muscular response to an extension-point having been ascertained for a given stimulus by registering the responses a few times at given intervals, the effect of interpolating a stimulation of a flexor-point in such an interval can be examined. The result of this interpolation is not infrequently practically to suppress the response of the E point for the time being, or to reduce one or other or both of its components (contraction and relaxation) very markedly (figs. 7, 8).

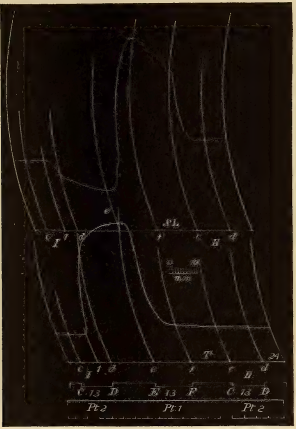

Fig. 7.

Experiment 8, *Macacus rhesus*, 29.1.12. E.F.E.—Record of two reactions evoked on stimulation of the same extension point (C—D, I and II) with an interpolated stimulation of a flexion-point (E—F). The first stimulation of the extension-point gives a marked extensor contraction and flexor relaxation. On its cessation there is a state of extensor tonus. This is broken down on stimulation of the flexion-point, and there is a marked flexor contraction, which persists after the cessation of the stimulus. The second application of the extension-point stimulation gives a certain degree of flexor relaxation (but not to such an extent as before) and no extensor contraction. This shows an approximation to reversal—that is to say, depression of both factors in the extension response.

Sometimes, however, the effect is an actual reversal of the usual response of the E point. The reversal may be partial or complete. In the former case the extinction of the contraction of the extensor may predominate. In

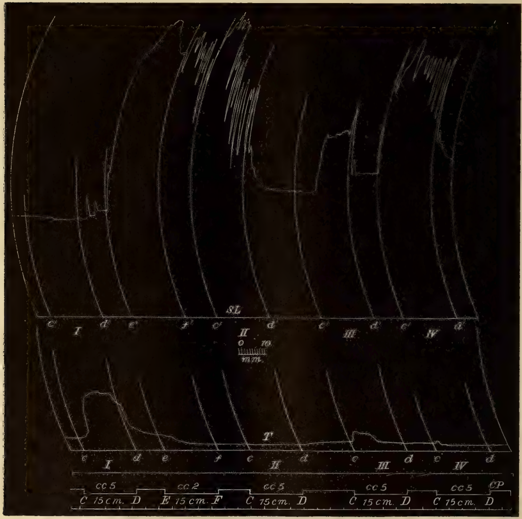

Fig. 8.

Experiment 11, *Callothrix*, 14.2.12. E.F.E.E.E.—In this record the conditions were the same as in the preceding figure, save that the stimulation of the extension-point was repeated thrice after the application of a stimulus to the flexion-point. The first E stimulus evoked a good extensor contraction. An F stimulus then gave a good flexor contraction and extensor relaxation. A second E stimulus then apparently increased a state of flexor epilepsy ; a third evoked (after the disappearance of the flexor epilepsy) a slight extensor contraction which soon disappeared during the application of the stimulus and in its disappearance was accompanied by a flexor contraction ; a fourth gave almost pure flexor contraction of an epileptiform type. This record demonstrates reversal of the extension reaction by inter-current flexion-point stimulation.

the full reversal contraction of the flexor replaces contraction of the extensor as the primary feature of the response (figs. 9, 10).

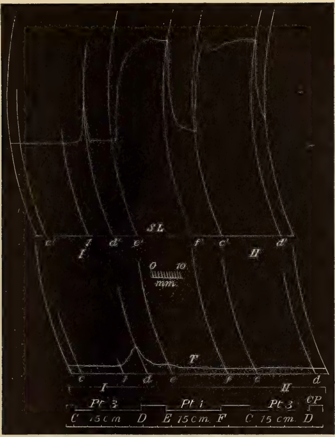

FIG. 9.

Experiment 9, *Macacus rhesus*, 9.2.12. E.F.E.—In this record a stimulus is twice applied to an extension-point (C—D, I and II), and between the two applications a flexion-point is stimulated (E—F). The first E stimulus gives a reaction of extensor contraction followed by flexor-contraction, so that at the end of the period of stimulation both the antagonists are in contraction at the same time. The F stimulus is then applied, and a subsequent E stimulus then evokes a reaction consisting of contraction of the flexor alone. This record demonstrated complete reversal of the extension reaction by interpolated stimulation of a flexion-point.

(b) *Changes in the Response of a Flexion-Point following Excitation of an Extension-Point.*

Just as repeated or strong excitation of a flexion-point often temporarily diminishes or suppresses the subsequent response of an extension-point, so conversely the response of a flexion-point is often temporarily lessened or retarded or rendered more difficult immediately subsequent to similar stimulation of an extension-point. The tendency of an E point thus to antagonise an F point is, however, in our experience, distinctly less marked than is that of an F point to suppress or to overcome an E point. Sometimes, however, the action of an F point is reversed after stimulation of an E point, and probably in consequence of the action of that point. This is shewn in the accompanying figure (fig. 11). Here the F point, although it caused contraction of the flexor four seconds before the stimulation of the E point, produces four seconds after that stimulation nothing but relaxation of that muscle,

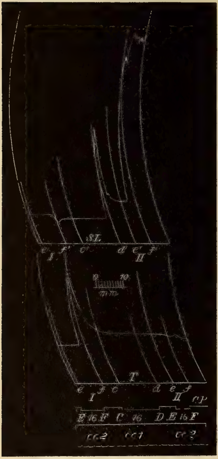

Fig. 10.

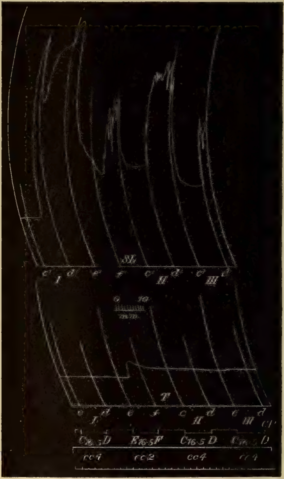

Fig. 11.

Experiment 14, *Macacus sinicus*, 21.2.12. E.F.E.—This record demonstrates the same phenomena of reversal as the preceding one, but the appearances are more complete. Here the first E stimulus evokes extensor contraction and flexor relaxation. The second E stimulus (applied after a flexion-point stimulus) gives flexor contraction and slight extensor relaxation—a clear case of complete reversal.

Experiment 14, *Macacus sinicus*, 21.2.12. F.E.F.F.—In this record an F stimulus (C—D, I) gives a good flexion reaction. An E stimulus (E—F) then gives a reaction in which there is exhibited at first augmentation of flexor tonus and then flexor relaxation accompanied by extensor contraction. A second F stimulus (C—D, II) then gives a relaxation of the flexor contraction in an epileptic state, as also does a third.

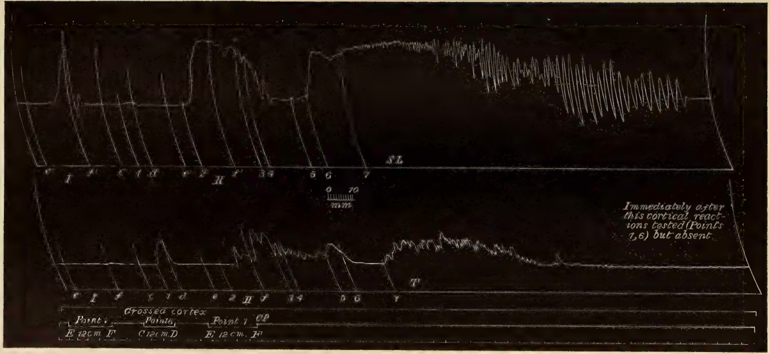

FIG. 12.

Experiment 12, *Troglodytes niger*, 16.2.12. F.E.F.—In this record an F stimulus (E—F, I) gives a good flexion reaction. An E-stimulus (C—D) then gives a distinct extension reaction, and a second F stimulus then gives a flexion reaction which is of greater extent and shorter latency than before. This is followed on cessation of stimulation by an epileptic discharge which is evidenced in both muscles and rapidly dies away. It is, however, soon succeeded by a second epileptic discharge which begins in the flexor muscle and is then accompanied by extensor relaxation. The extensor soon, however, contracts and at the same time the discharge becomes discontinuous—clonic. The beats become slower and of greater extent and then suddenly cease. This was followed by " post-epileptic " inexcitability.

and similarly again four seconds later. Here, however, it must be noted that the flexor at the time of the second and third repetitions of the F point stimulation is exhibiting epileptoid contraction.

Occasionally, as in fig. 12, the interpolation of brief stimulation of an E point is followed by a somewhat increased response of the F point, but in such cases it is, of course, difficult to decide whether this slight increase may not be due to the precurrent stimulation of the F point itself which is necessary as a control at the beginning of the observation.

V. INFLUENCE OF CERTAIN AFFERENT NERVES UPON THE REACTION OF THE CORTICAL POINT.

As the antagonistic pair of muscles under observation actuated the fore-arm, it seemed that stimulation of an afferent nerve of the fore-arm might influence the response of the observed muscles to stimulation of the cortical points. That in the chloratised rabbit electrical stimulation of the skin of the fore paw and of the fore limb cortex of the opposite hemisphere usually summate has been shown by Exner.* And, recently, A. Uchtomsky has carefully examined in the dog the mutual influence of the peroneal and other limb nerves on the responses of the motor cortex for the limb, both ipsilateral and contralateral. He finds marked mutual interaction, both facilitatory and inhibitory, and that "im allgemeinen bei gegenseitiger Einwirkung der kortikalen und der reflektorischen Innervationen der Beine die ersteren eine weit überwiegende Rolle spielen."

In our observations *nervus ulnaris* in the fore-arm was the nerve chosen and it was stimulated by faradisation. It was ascertained that in the decerebrate preparation (monkey) stimulation of this afferent nerve usually evokes contraction of the flexor and inhibitory relaxation of the extensor when that nerve is the ipsilateral one, and conversely evokes contraction of the extensor and inhibitory relaxation of the flexor when it is contralateral. This is in accord with the general rule previously formulated chiefly from cat and dog.

It was expected, therefore, that when working with the muscles of the left elbow the stimulation of the left ulnar nerve would reinforce the effect of stimulation of an F point of the right hemisphere. And this was found to be the case (fig. 13). Conversely, stimulation of right ulnar, as might be expected, was observed to diminish the effect of the stimulation of the right hemisphere's cortical F point (fig. 14). But the tracing here reproduced illustrates a further result, namely, that the cortical F-point on restimulation after the peripheral nerve stimulation may produce relaxation instead of contraction of the flexor muscle—that is, may shew reversal of its previous action. Whether such reversal is referable to the epileptoid discharge induced or to the influence of the precurrent peripheral nerve stimulation remains, however, an open question.

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* 'Pflüger's Archiv,' 1882, vol. 28, p. 502.

Turning to the influence of the peripheral nerve stimulation upon the response of the cortical E point, this influence has been marked in the case of the ulnar nerve of the ipsilateral side. The stimulation of this nerve, while reinforcing the action of the F point (*cf. supra*), seems to antagonise that of the E point. Sometimes temporarily the reaction of the E point is practically suppressed after the reaction from the ipsilateral ulnar nerve (fig. 15). Sometimes E's reaction is absolutely reversed; this we have seen when during an ipsilateral ulnar stimulation the E point has been excited and instead of

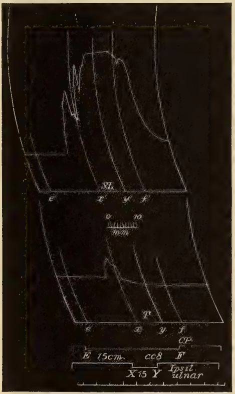

Fig. 13.

Experiment 15, *Callothrix*, 5.2.12.—In this record a cortical point which was giving primary flexion was stimulated (E—F), and during that stimulation another stimulus (X—Y) was applied to the ipsilateral ulnar nerve. The cortical stimulus just before the application of the nerve stimulus had produced a contraction of both the antagonists. The effect of the nerve stimulus was to augment the contraction of the flexor and abolish that of the extensor.

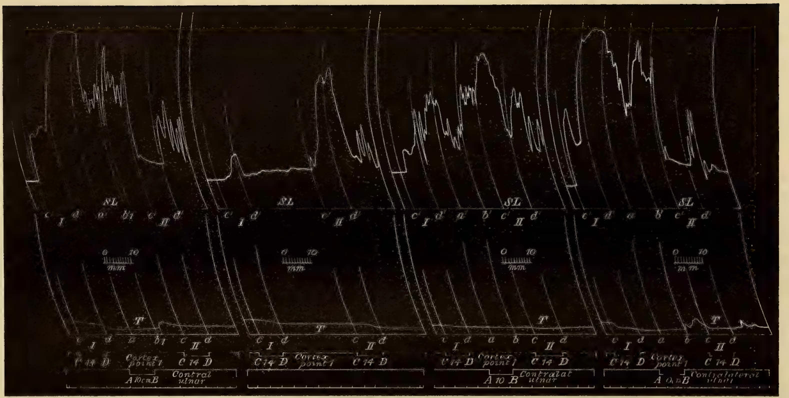

Fig. 14.

Experiment 7, *Macacus rhesus*, 26.1.12.—This figure consists of four records, and demonstrates the effect upon an F-point reaction (C—D) of stimulation of the contralateral ulnar nerve (A—B).

In the first record the F stimulus is first applied and gives a flexion reaction which persists after cessation of stimulation. The stimulation of the contralateral ulnar gives relaxation of this contraction, and a second F stimulus then applied gives also relaxation—the flexion reaction therefore being reversed.

In the second record the F point is twice stimulated at the same interval as in the first, but with no interpolated nerve stimulus. The second stimulus gives an augmented flexion reaction, so that the reversal in the first record can hardly be due to repetition of stimulation.

The third record is a repetition of the first with the same strength of nerve stimulus. There is here no reversal at the second F stimulus, but augmentation of flexion.

In the third record the nerve stimulus is increased in strength and there is again a marked reversal of the flexion reaction. In each of the two latter records there is an epileptic discharge, and it therefore looks as if here the reversal was not due to the presence of the epilepsy but to the nerve stimulus.

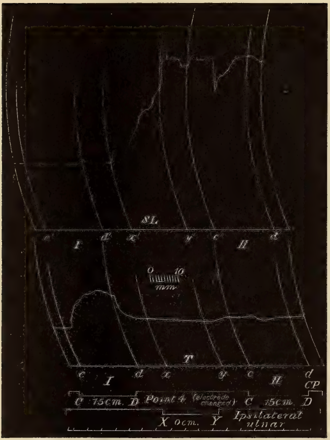

FIG. 15.

Experiment 9, *Macacus rhesus*, 9.2.12.—In this record a cortical E point is twice stimulated with an interpolated stimulation of the ipsilateral ulnar nerve. The first E stimulus gives a good extension reaction. The ipsilateral ulnar nerve then gives a flexion-reflex. At the second repetition of the E stimulus there is little movement in the extensor tracing, and the state of maintained flexor contraction is not markedly interfered with, there being only a transient and small relaxation. This shows depression of the E point reaction.

causing relaxation of the flexor's contraction has increased that contraction, although on being restimulated a few seconds after the cessation of the ulnar reaction it has caused its usual flexor relaxation (fig. 16).

Taken together, the effect of the stimulation of the ipsilateral afferent nerve, which in the decerebrate preparation is to contract the flexor and inhibit the extensor of its own limb's elbow, is with the cerebrum intact to augment the action of the cortical point for elbow flexion and to reverse the action of the cortical point for elbow extension to elbow flexion. This indicates that the activity of the local peripheral afferents may be a factor of importance in determining what one of us has termed the neural balance

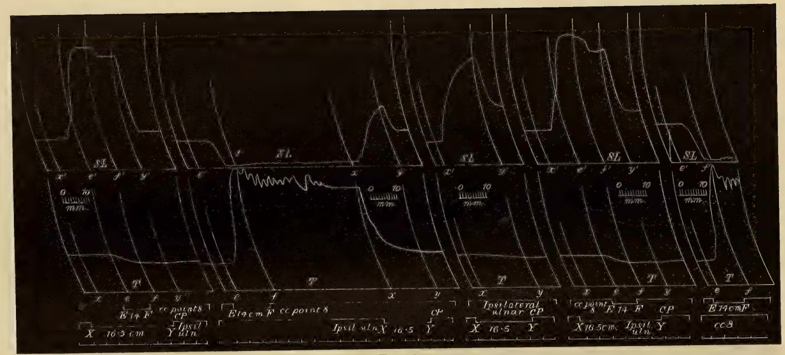

Fig. 16.

Experiment 15, *Callothrix.* 4.3.12.—This figure consists of five consecutive records.

In the first of these a stimulus is first applied to the ipsilateral ulnar nerve, and during this the E point is stimulated. After the commencement of the E stimulus there is seen to be a marked augmentation of the flexor contraction. This slightly decreases when the E stimulus is stopped, although the stimulation of the nerve is still in process.

The second record, taken a few seconds after the first, shows that the E stimulus alone gives an extensor contraction and flexor relaxation. This is then followed, after cessation of stimulation, by a state of maintained extensor contraction which is epileptiform in character. During this a stimulus applied to the ipsilateral ulnar nerve gives a flexion reflex.

The third record (1 min. later) shows a flexion reflex, evoked by stimulation of the ipsilateral ulnar nerve. It will be seen that this is of greater extent than that evoked during the epileptiform discharge of the extensor.

The fourth record (1 min. later) is a repetition of the first, and again shows—on E-point stimulation—augmentation of the flexor contraction evoked by stimulation of the ipsilateral ulnar nerve. If the outline of this and the first flexor tracing be compared with the outline in the third record, it will be observed that it is similar up to the point at which the E point is stimulated (ordinates e') and there markedly steepens.

The fifth record (1 min. later) shows again the effect of stimulation of the E point alone—a marked extension reaction.

of the nervous system throughout a whole series of centres dealing with the locality whence the excited afferents proceed.

The mutual influence of the peripheral stimulus (afferent nerve) and cortical point on each other's responses depends largely on the relative intensities of their stimulations. Thus the effect of the cortical E point can be wholly reversed by the stimulation of the afferent nerve with certain intensities of stimulation. But with stronger stimulation of the E point its own usual effect can be made to break through, and for a time wholly suppress that of the afferent nerve. There are intermediate degrees in which the E point's effect, although initially reversed, quickly returns subsequently to its own usual character and subtracts from that of the peripheral nerve and even wholly suppresses it.

VI. THE EFFECT OF CONCURRENT STIMULATION OF TWO CORTICAL POINTS ANTAGONISTIC TO EACH OTHER.

In some of the experiments the stimulation apparatus has been so arranged as to allow of faradisation of two cortical points at the same time. We have

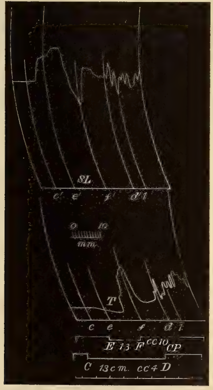

FIG. 17.

Experiment 15, *Callothrix*, 4.3.12.—In this record a cortical F point (C—D) is stimulated, and during the period of stimulation a cortical E point (E—F) is concurrently stimulated. It will be seen that during the F reaction the E stimulus produces a flexor relaxation and a late extensor contraction.

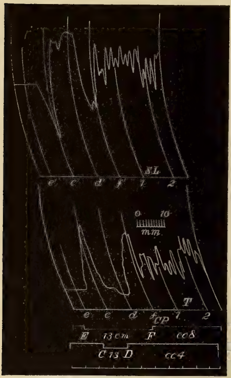

Fig. 18.

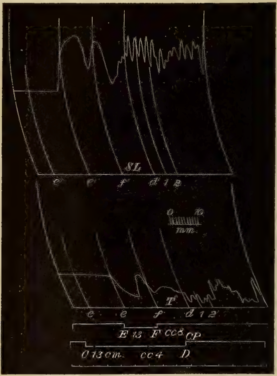

Fig. 19.

Experiment 15, *Callothrix*, 4.3.12.—From the same experiment as the last, but here the E stimulus (E—F) is first applied, and during its application the F stimulus (C—D) is given. Here the extensor contraction is cut down during the F stimulus, and the flexor relaxation gives place to contraction. At the cessation of the F stimulus the flexor relaxation and extensor contraction are re-established.

Experiment 15, *Callothrix*, 4.3.12.—In this record the F stimulus is first applied, and the E stimulus is applied later during its application. The E stimulus here does not so markedly antagonise the F reaction as in fig. 17. The extensor tonus is re-established and the flexor relaxes, but not to the level which obtained before the first stimulus was applied.

in this manner sought to see the effect on the reactions of one point produced by intermittent stimulation of its antagonistic point. Figs. 17, 18, 19, 20 illustrate the effect. The F point stimulated intermittently during the stimulation of an E point may antagonise that action and establish its own. The E point acting during the action of an F point may similarly break through the F point response and establish its own effect. But there is evidence that in such cases the intercurrent point does not get its full play, in other words that there is some algebraic summation of the two opposed actions.

When the stimulations of E point and F point are nearly balanced there is a tendency for whichever of the two is applied subsequently to break through the effect of its opponent.

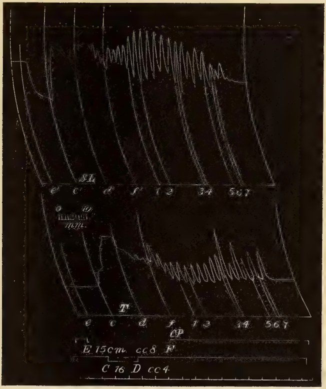

Fig. 20.

Experiment 15, *Callothrix*, 5.3.12.—Here the E point (E—F) on stimulation gives flexor relaxation, and concurrent stimulation of the F point (C—D) reverses this to flexor contraction, but at the same time there is an extensor contraction, so that both the antagonists are in contraction at one and the same time. The cessation of the E stimulus is followed by an epileptiform discharge in both the antagonists. An examination of the ordinates numbered 1—7 on the two muscle tracings shows that the beats in these are reciprocal. Thus ordinate 5 on the extensor tracing cuts the lowest point in a beat, while the corresponding ordinate 5 in the flexor tracing cuts the top of a beat. Thus near this point relaxation during a beat in one muscle was accompanied by contraction during a beat in the other.

VII. Post-central Convolution.

We have on a number of occasions found stimulation of points in the post-central convolution near the lower end of the intra-parietal sulcus modify the epileptoid after-discharge following stimulations of points in the arm-area of the pre-central convolution. Points in the post-central convolution when stimulated immediately after cessation of a stimulation of a pre-central gyrus are frequently able to evolve some return of the movement just previously elicited from the pre-central convolution, producing, so to say, an echo of the pre-current response, but this dies out usually after the first repetition.

VIII. After-Discharge subsequent to the Response of a Cortical Point.

(a) *Tonic After-Discharge.*

Among the forms of after-action (attached to the responses) evoked by cortical stimulations one commonly met with is what may be termed "tonus remainder." This was noted by Bubnoff and Heidenhain in certain degrees of morphia-action in the dog; they speak of the phenomenon as contracture.* It resembles closely the shortening reaction observable in reflexes evoked during decerebrate rigidity. It differs from that, however, (1) in being less complete, *i.e.*, in less completely maintaining the full amplitude of the contraction on which it supervenes, and (2) in appending itself to flexor as well as to extensor contractions (figs. 3, *a*, *b*; 4; 5; 16), whereas in decerebrate rigidity it is only the extensor contractions which exhibit the post-stimulatory continuance. Conversely, just as in decerebrate rigidity a lengthening reaction appends itself to and prolongs a reflex relaxation, so too after cortical inhibitory relaxations a relaxation remainder persists as a common after-effect. Here also the appended remainder does not so nearly equal the full relaxation it follows as it does in decerebrate rigidity. And here too it appends itself in the cortical reactions both to flexor and to extensor effects: whereas in decerebrate rigidity it appends itself to extensor alone.

The degree of tonus in the antagonistic muscles is reciprocal; and the influence of a cortical point on the tonus as seen in the effect of stimulation of the point is also reciprocal (figs. 2, 3, 4).

(b) *Epileptoid After-Discharge.*

Epileptoid after-discharge of limited distribution has occurred not infrequently in our experiments. It has been frequently observable that the epileptiform clonic discharge following upon stimulation of an F point or an E point is confined as far as the muscles under examination by the myograph are concerned to one of the antagonists (figs. 6, 8, 11, 14, 16). We have more than once seen that an epileptoid discharge in the flexor muscle following on stimulation of a flexor cortical point is increased by subsequent stimulation of an extension cortical point (fig. 8), while restimulation of the flexion cortical point diminished the epileptoid discharge during the period of the stimulation (fig. 11).

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* 'Pflüger's Archiv,' 1882, vol. 26, p. 136.

Although the epileptiform clonus has often been confined to one of the antagonistic muscles, either flexor alone or extensor alone, sometimes it has appeared in both these muscles together (figs. 12, 17, 18, 19, 20); the effect has then been sometimes exquisitely reciprocal (fig. 20), but not uniformly so, a sudden break in the rhythm of the clonus of one muscle occasionally disturbing the mutual relation.

Subsequent to a partial epileptoid after-discharge we have frequently found the area of cortex whence the discharge was initiated irresponsive for a time (post-epileptic exhaustion of François-Franck and Pitries). In our experiments this was particularly noticeable in the case of the cortex of the anthropoid ape, the chimpanzee (fig. 12), and more marked than in the case of the smaller monkeys (macacus) which we have used.

(c) *Rebounds.*

In his interesting monograph on the reactions of the motor cortex of the dog, A. de Uchtomsky* has called attention to rebound effects, both excitatory and inhibitory, following cortical stimulations, and furnishes excellent illustrations of them. From his work we gather in comparing with it our observations on the monkey that cortical rebounds tend to occur more frequently in the dog than in the monkey.

We have seen on several occasions that a cortical stimulus which produces inhibition of one of the muscles under observation is, on its cessation, followed by a contraction of the inhibited muscle. This contraction appears to be a rebound contraction similar to the rebound contractions which are exhibited so frequently by extensor muscles after an inhibitory reaction in the decerebrate preparation, and more rarely in purely spinal preparations. In the experiments with the cortex we have met with examples of rebound contractions both in the extensor and in the flexor muscles (figs. 21, 22). They are not, however, in our experience nearly so often met with as in the reflex reactions of the decerebrate preparation.

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* 'On the Dependence of Cortical Motor Reactions upon Central Associated Influences (Russian), Moscow, 1911.

Fig. 21 = Expt. 8.

Fig. 22 = Expt. 15.

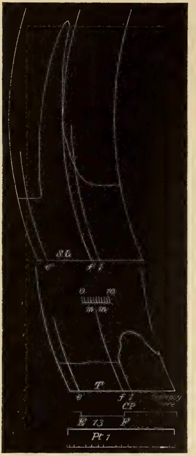

Fig. 21.

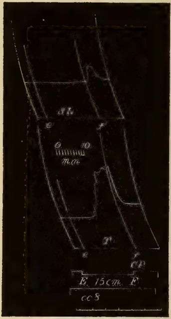

Fig. 22.

Experiment 8, *Macacus rhesus*, 29.1.12.—Record of the stimulation of a cortical flexion-point (E—F). The cessation of stimulation is seen here to be followed at an interval of about 1·5 seconds by an extensor rebound contraction.

Experiment 15, *Callothrix*, 5.3.12.—Record of the stimulation of an extension-point (E—F). The cessation of stimulation is here followed at a short interval by extensor relaxation and a small, but clear, flexor rebound contraction.

IX. CONCLUSIONS.

The cortical effects, as examined in this pair of antagonistic muscles, are clearly very complex and composite. Variable latencies, sometimes of great duration; long and varied after-actions, some tonus-like and others rhythmically clonic, others in the nature of rebounds; mutual relations of great diversity between the reactions of the muscle-pair during actual stimulation; all these, along with other characters in the records not easily dealt with in a short communication, impress upon the observer the high complexity of the cortical as compared with decerebrate or purely spinal reactions.

A phenomenon of outstanding prominence in the results has been that of inhibition. In our records it appears more prominent than excitation itself. Often it manifests itself along with excitation as a reciprocal effect, also, however, it occurs without obvious concomitant excitation of the antagonistic muscle, and, so far as our experience at present goes, more often than does the contrary phenomenon—namely, excitatory contraction without concomitant inhibitory relaxation of the opponent. This feature of the records inclines us to suppose that the inhibitory and excitatory effects respectively—although with great frequency reciprocally yoked together under cortical stimulation—are, as cortical reactions, nevertheless, to a considerable extent independently exhibitable. In regard to this, however, it has, of course, to be remembered that our observations have of necessity been confined to a particular muscle-pair as a sample of the whole limb musculature. Were it possible to survey contemporaneously more of the several muscles of the limb, the wider field of observations might possibly reveal closer concomitance between the two effects. In some cases the records exhibit concomitant increase of contraction in both of the component muscles; in such cases it may well be that double reciprocal innervation is at work—as has been pointed out, that phenomenon may quite reasonably be expected as a reaction from a cortical point. But it must be admitted that as yet no experimental proof has been given that it actually is so.

In directing attention to the frequency of temporary changes in the effect exerted by a cortical point upon an antagonistic muscle-pair, and in shewing that these changes not rarely reach actual reversal of the sense of the movement, we would not be understood as impugning the general regularity of the results which such cortical points yield when appropriately examined. The same cortical point, returned to after a period of quiescence, will usually, time after time, yield approximately the same result on the muscle-pair with considerable consistency. What we desire to stress in regard to the reaction of the cortical point on the antagonistic muscle-pair is the influence of shortly pre-current excitations, both of itself and of other points, and of afferent channels. And in this respect the functional instability of some cortical points seems to be greater than that of others. The instability is great enough to be easily demonstrable under narcosis as in our experiments. It may well be even greater in normal conditions without narcosis. Indeed, the frequence of reversal as a phenomenon attaching to the reactions of points in the motor cortex suggests that one of the functions of the cortex may be performance of reversals, and that the greater predominance of reversal under cortical than in purely spinal or decerebrate reflexes is because reversal is one of the specific offices of the cortex cerebri.

The Factors in Rhythmic Activity of the Nervous System.[*]

By T. Graham Brown (Carnegie Fellow).

(Communicated by Prof. C. S. Sherrington, F.R.S. Received April 11,—
Read May 16, 1912.)

(From the Physiology Laboratory of the University of Liverpool.)

I. *Introduction.*

In a communication upon the intrinsic factors in progression presented to the Society in July of last year[†] the author drew the conclusion that the rhythm of progression is of central origin. In then enquiring into the nature of that central origin he suggested that the movements are determined by a balance between equal and opposite states of excitation—flexion producing and extension producing.[‡]

An obvious line of research is thus presented by the possibility that reflex stimuli which normally evoke opposite reactions (flexion and extension), that is, opposite states (of excitation and inhibition) in one and the same centre, when applied at the same time and with appropriate strengths of stimulus may give a rhythmic response.

When that communication was made such experiments had actually been performed and had yielded positive results. The facts were not recorded because it was intended to give them in greater detail at a later date. They have been embodied in a thesis presented to the University of Edinburgh; but as some time must pass before they can be published in full it is desired to give a brief account of them here.

II. *Methods.*

The muscles selected for examination were *tibialis anticus* and *gastrocnemius* —a flexor and an extensor of the ankle. These were prepared in a manner already described,[‡] and their movements in response to stimulation of the ipsilateral and contralateral long saphenous nerves examined. The muscles wrote, by means of levers attached by threads to their tendons, upon the blackened surface carried by a kymograph.

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[*] The expenses of this research have been defrayed by a grant from the Carnegie Trust.

[†] 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 308.

[‡] 'Quart. Journ. of Exp. Physiol.,' 1911, vol. 4, p. 331.

III. *Rhythmic Responses to Simple Stimuli.*

The simple stimulus usually evokes a muscular response characterised by contraction of one muscle and relaxation of its antagonist.

Occasionally in the ipsilateral flexion-reflex the flexor contraction dies down during the process of the stimulus and at the same time an extensor contraction appears. At the cessation of stimulation there may be a flexor rebound. In a similar manner the contralateral extension-reflex occasionally exhibits contraction of both the antagonists. Tracings demonstrating these points have already been published.*

A third type of reaction is seen in the rhythmic responses which are occasionally evoked.

In the low spinal preparation these are rare but may be evoked after a mechanical stimulation of the upper part of the lumbar portion of the divided spinal cord. This often evokes a state of maintained extension, and a flexion-reflex then super-added may produce a rhythmic reaction. This resembles the " balanced " phase of the progression reaction obtained on rapid division of the spinal cord.†

So too may a reflex stimulus applied shortly after such a rapid division—when the phenomenon is in the third phase of maintained extension.

In the decerebrate preparation there is a much greater tendency to the appearance of rhythmic reactions in response to simple stimuli. Tracings in which this is evident have already been published.* These may appear when, as was described above in the case of the low spinal preparation, there is no such artificial background of maintained extension. But it must be remembered that in the decerebrate preparation there is always the background of decerebrate rigidity—that is, a maintained extension, and there is usually evident contraction of both antagonists. These rhythmic reactions resemble the movements of progression and are reciprocal in the two muscles. It happens very rarely that they resemble the movements of the scratch-reflex. Both muscles contract together, and the " beats " seem to occur as relaxations of these contractions. They occur alternately in the two muscles, the point of maximum relaxation in one corresponding to the point of maximum contraction in the other.

Another rhythmic response in the simple reflex is the " rhythmic rebound " already described by the author.* This occurs especially in the decerebrate preparation and after cessation of a stimulus. The movements undoubtedly resemble those of progression.

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* 'Quart. Journ. of Exp. Physiol.,' 1911, vol. 4, p. 331.

† 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 308.

IV. *Rhythmic Responses to Compounded Stimuli.*

Sherrington* has pointed out that weak inhibition is often accompanied by tremor. In this paper he refers to the reflex inhibition of the state of decerebrate tonus by a stimulus which produces a flexion-reflex. Dr. Forbes has informed me that he observed, some time since, a similar tremor on pitting two opposite reflex stimuli one against the other. Sherrington has also shown that the tremor is reciprocal.†

In the present experiments two opposite reflex stimuli were also pitted against one another, but the rhythmic response which was evoked cannot be described as "tremor." It is far more akin to the rhythmic movements of the act of progression.

The results were obtained in the low spinal and in the decerebrate preparations. In both kinds of preparation it was found in some cases that although a simple contralateral stimulus when applied alone gave a response of steadily maintained extensor contraction, and although a simple ipsilateral stimulus similarly applied gave pure maintained flexor contraction, yet when these were applied together the response became a rhythmic alternation of flexion and extension.

Thus if the contralateral stimulus was first applied it evoked a maintained contraction of the extensor. If then an ipsilateral stimulus was applied the extensor response ceased to be steady and became rhythmic. On then taking the ipsilateral stimulus off again the extensor response again became steady (figs. 1 and 2).

In a similar manner the steady maintained flexion evoked by the ipsilateral stimulus might become rhythmic on the application of the contralateral. In some cases there was a contraction of both muscles and each of these was rhythmic. The rhythmic movements were reciprocal—that is to say that one rhythmic "beat" was in process of relaxation while the synchronous "beat" in the other muscle was in process of contraction (fig. 3).

These phenomena were also seen in muscles after the division of their proprioceptive afferent nerves.

In all these cases the rhythmic phenomenon occurs only when the strengths of stimuli bear a certain fixed relationship to one another. If one stimulus is progressively increased and compounded with the fixed opposite stimulus a rhythm present gradually decreases in distinctness, and finally disappears.

The rate of the rhythmic movements usually lies between one and two beats per second.

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* 'Quart. Journ. of Exp. Physiol.,' 1908, vol. 1, p. 67.

† 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 249.

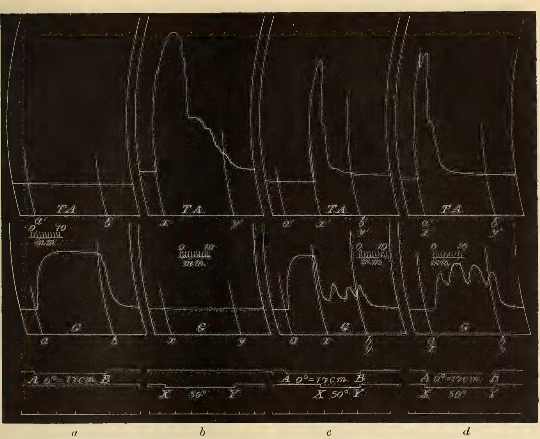


Fig. 1.

Experiment 30, 31.3.11.—Ordinary decerebrate preparation, a series of four reactions.

In the first of these (*a*) a simple contralateral stimulus is applied between the marks A, B, of the signal line (ordinates *a*, *a'* ; *b*, *b'*). This evokes a simple reaction of maintained extensor contraction—the crossed extension-reflex.

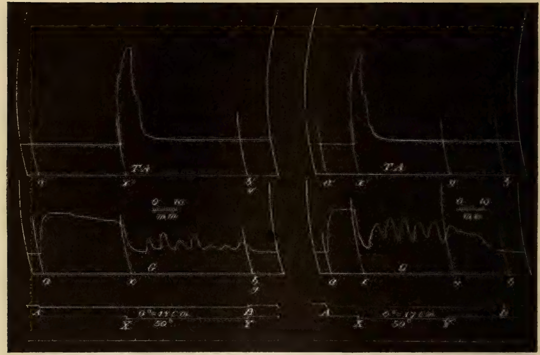
In the second of these (*b*) a simple ipsilateral stimulus (signal X, Y—ordinates *x*, *x'* ; *y*, *y'*) evokes a simple reaction of maintained flexor contraction—the ipsilateral flexion-reflex.

In the third of these (*c*) the contralateral stimulus is first applied (A—B) and then evokes maintained extensor contraction. During this stimulus an ipsilateral one is added (X—Y). This gives a smaller flexor contraction than in "*b*," and at the same time there is a rhythmic movement in the extensor tracing.

In the fourth record (*d*) both stimuli are applied at once, and the extensor movement—the latency of which is increased—is rhythmic from the first.

The strengths of the ipsilateral and of the contralateral stimuli remain the same throughout this series.

In all the figures the upper tracing is that of the flexor—*tibialis anticus*, and the lower that of the extensor—*gastrocnemius.* The upper signal line is that of contralateral stimuli, the lower that of ipsilateral. The lowest line marks seconds and millimetre scales are reduced in proportion with the tracings. Corresponding ordinates on the tracings mark the beginnings and terminations of the stimuli, and the rise of the levers denotes contraction of a muscle—the fall relaxation.



e *f*

Fig. 2.

Experiment 30, 31.3.11.—A continuation of the last figure. Two reactions.

In the first of these (*e*) the contralateral stimulus is applied for some time (about six seconds) before the ipsilateral stimulus is added. They then run together for about eight or nine seconds. During this time the extensor movements—which before were maintained—are rhythmic.

In the second reaction (*f*) the ipsilateral stimulus is applied earlier, but is taken off again before the termination of the contralateral. Here the extensor contraction is maintained before the commencement and after the termination of the ipsilateral stimulus, but when both stimuli run together it is rhythmic.

V. *Other Forms of Rhythmic Movements.*

The author has previously described movements of progression which occur under narcosis in the rabbit.* Similar movements have been seen in the guinea-pig under suitable circumstances. These in their rhythm seem to bear the simple relationship of 1 : 4 to the rhythm of the "beats" of the scratching movements which occur under narcosis in the guinea-pig. In the rabbit the relationship seems to be commonly 1 : 2.

Similar movements also occur in the cat. These are of additional interest, in that they may be observed in the same individuals from which records of the other movements of progression, provoked in other ways—*e.g.*, reflex, central on cutting spinal cord, etc.—were subsequently obtained.

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* 'Quart. Journ. of Exp. Physiol.,' 1911, vol. 4, p. 151.

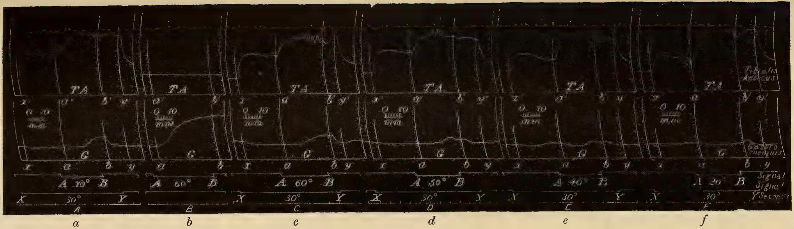

Fig. 3.

Experiment 25, 15.3.11.—Ordinary decerebrate preparation, a series of six reactions.

In this series the second (*b*) is a reaction of pure maintained extensor contraction in response to a simple contralateral stimulus. The other reactions are all in response to compounded ipsilateral and contralateral stimuli. The strength of the ipsilateral stimulus remains constant, but that of the contralateral is progressively increased. It will be noticed, for instance, in the first reaction, that before the application of the contralateral stimulus the ipsilateral gives a reaction of pure maintained flexion.

In the first reaction (*a*)—as well as in the succeeding ones—the application of the contralateral stimulus gives an *augmentation of the flexor contraction.* This is remarkable, as that stimulus alone (*b*) gives extensor contraction. This is soon followed (in "*a*") by a contraction of the extensor, the latency of which is great. At this point both muscles are contracting together, and a rhythmic movement is seen here in the flexor tracing. A slighter rhythmic movement is present in the extensor tracing.

In the third reaction (*c*) the strength of the contralateral stimulus has been increased. The rhythmic phenomenon in both muscles is more marked and a minute examination shows that it is reciprocal—so that the contraction phase in one is accompanied by a relaxation phase in the other.

In the fourth reaction (*d*) a still stronger contralateral stimulus gives an increased flexor augmentation. The rhythmic phenomenon is less marked, and so too is the extensor contraction.

In the fifth reaction (*e*) a yet stronger contralateral stimulus gives a greater augmentation of the flexor contraction. This is still less rhythmic. In the extensor tracing there is first a marked relaxation and then a slight contraction.

In the final reaction (*f*) a stronger contralateral stimulus gives a greater augmentation of the flexor contraction. This is now arrhythmic, and there is almost no evidence of extensor contraction—only of relaxation.

(At the end of this series a pure contralateral stimulus gave a reaction of pure maintained extensor contraction as before.)

The rate of the rhythm of these movements is about one to two beats per second. Under normal circumstances it has been observed to be as slow as 0·6 beat per second, and as fast as 2·5 beats per second. In asphyxia the beats become markedly faster (besides changing in other ways), and their rate may then rise to one of 3·3 beats per second, or even more.

The movements have been registered as they occurred in the isolated muscles as well as in the intact hind limbs. They have then an appearance exactly similar to that of the rhythmic movements seen in some of the other types of progression examined.

Another form of progression is that obtained on electric stimulation of the cut surface of the spinal cord. This also closely resembles the other forms in appearance and in rate of rhythm. If the strength of the exciting stimulus be progressively increased the rhythmic reaction becomes less well marked and finally disappears, the reaction becoming one of pure maintained contraction.

VI. *Comparison of the Different Forms.*

When one or more of these different forms of progression—in narcosis; on electrical stimulation of the spinal cord; on rapid section of the lower thoracic spinal cord; in response to simple peripheral stimulation, either as a direct response or as a "rebound"; and in response to compounded peripheral stimuli—occur in the same individual, the general appearances of the different reactions are seen strikingly to resemble one another. Even peculiarities present in one variety may be present in the other. Thus, in one instance, the rhythmic rebounds ensuing on cessation of a reflex stimulus presented the peculiarity that the "beats" were arranged in groups of two, the second of which was smaller than the first. The "beats" of the progression movements which were evoked subsequently on section of the spinal cord also presented this peculiarity.

VII. *The Conditioning of Rhythmic Movements.*

These experiments seem to shew that rhythmic movements similar to those of progression may be evoked on compounding two antagonistic excitabilities, that is, two antagonistic states of excitement and inhibition in each centre. They therefore seem to confirm the suggestion previously put forward that this is the method of the determination of progression.

In the first place the direct compounding of antagonistic excitations—seen in the compounding of stimuli which evoke antagonistic reflexes—may in certain cases produce the phenomenon.

Secondly, the application of a simple stimulus may also do so. But in these cases there is really a compounding of two antagonistic excitations—for there must be a background of maintained extension, as in the decerebrate condition, or as in the experiments in the low spinal preparation in which the mechanical stimulation of the spinal cord produced the maintained extensor contraction.

Even in the case of the simple reflex there may be a compounding of two antagonistic excitations apart from any background present. This may be the case if the afferent nerve can activate (excite) both centres (extensor and flexor), although not with equal effect. Such a condition may occur if the nerve split in the spinal cord and one part goes to each centre to excite it. Or it may occur if the afferent nerve stimulated in these experiments contains fibres some of which pass to one of the antagonistic centres and some to the other.

That such a double reciprocal innervation may occur even with a simple stimulus—that is, a stimulus applied to a single afferent nerve—seems to be shewn by the fact that sometimes contraction of both muscles may occur during the process of the stimulus.

Another point which seems to lend support to this view is the occurrence of flexor and of extensor "rebound contraction after excitation." If rebound be a phenomenon associated with a state of inhibition it must seem that even in the efferent centre of the contracting muscle there is a certain factor of inhibition produced by the peripheral stimulus.

It may be stated here that the corresponding phenomenon "flexor (or extensor) rebound relaxation after inhibition," which had not been observed when the above phenomenon was described,* has since been seen by the author in the case of the extensor muscle. An ipsilateral stimulus which evoked flexor contraction, and along with that a well-marked extensor relaxation, at the cessation of its stimulation was followed by an additional extensor relaxation.

VIII. *Theories of Rhythmic Activity.*

It is not intended here to discuss these in detail. It may be remarked, however, that certain evidence given in this paper seems to be directly opposed to three well-known theories.

In the first place the occurrence of relaxation in a rhythmic phenomenon may markedly precede the occurrence of the synchronous contraction of the antagonistic muscle.*† This phenomenon would not be possible if the state of inhibition was conditioned by a phenomenon of "drainage" as suggested by McDougall,* and a theory of rhythmic movement founded upon this conception can hardly be a correct one.

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* 'Quart. Journ. of Exp. Physiol.,' 1911, vol. 4, p. 331.

† 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 308.

In the second place the intrinsic factor in the production can hardly be a metabolic one. That is to say, that it cannot be intrinsically conditioned by the metabolic activity in the moto-neurones. Were this the case, a rhythmic response would follow each expression of activity of the centres. But this is not the case. The application of a continuous stimulus to a single peripheral afferent nerve usually evokes a response in which the one muscle contracts continuously—that is (as regards such coarse rhythms as those of the scratch-reflex or of progression), arrhythmically. But two such stimuli, which evoke antagonistic activities when compounded together, may give a rhythmic response. The intrinsic condition of the rhythmic phenomenon lies not in the efferent centres themselves, but in some property of their inter-dependence and inter-relationship.

The third theory to account for rhythmic activity is that which assumes a peripheral self-generated antagonistic stimulus. That this is not a correct one is shown by the occurrence of the phenomenon after the de-afferentation of the muscles.†

The theory suggested to account for rhythmic activity is the following: The cell-bodies and their processes of the efferent neurones of the antagonistic muscles form centres which mutually inhibit each other. A stimulus which falls upon one will therefore through it inhibit the other. But if this inhibition reduce the activity of the second centre, that will inhibit the first less, and so the process will proceed until there is a limit set to this "progressive augmentation of excitation."

But if a stimulus fall more or less equally upon the two antagonistic centres—or if two equal stimuli fall upon them—that which is most activated will have its excitability increased by "progressive augmentation" up to a certain point. The limit may be set by a process of inhibitory fatigue. If this proceeds the balance will turn in the opposite direction, and there will be a progressive augmentation of excitation in the other centre until it too reaches its limit, when the process will set in in the other direction again. In such a scheme there is, however, no explanation of the occurrence of inhibition before excitation in time. It is not difficult to overcome the difficulty by postulating a pair of "interposed centres" between the afferent neurone and the efferent centres, and by supposing that these too mutually inhibit, and that, in addition, they inhibit the crossed primary centres. It will be observed that in this scheme no explanation of the nature of the inhibitory process is given.

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* 'Brain,' vol. 26, p. 153.

† 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 308; 'Quart. Journ. of Exp. Physiol.,' 1911, vol. 4, p. 331.

Summary.

1. In the case of simple peripheral stimulation the reflex response is sometimes of a rhythmic character. It then resembles the rhythmic movements of progression which sometimes occur in the cat during the state of narcosis.

2. The rhythmic reaction occurs sometimes during the application of the stimulus and sometimes at its cessation as a rhythmic rebound.

3. In the low spinal preparation the rhythmic reaction during stimulation is rare. It is seen almost alone after the production of an artificial state of maintained extension by mechanical stimulation of the spinal cord, and in response to flexion-producing stimuli. Here it looks as if the reaction was conditioned by the pitting against one another of two opposite activities.

4. In the decerebrate preparation of the cat, rhythmic responses may appear in reaction to flexion-producing stimuli. Here there is already, in the decerebrate rigidity, a state of maintained extension.

5. Both in the normal and in the de-afferented conditions of the low spinal and decerebrate preparations the compounding of reflex stimuli, which, taken singly, produce opposite reactions (flexion and extension), sometimes results in a rhythmic reaction. The rhythmic movements may occur in both muscles, and they are then reciprocally alternate.

6. These rhythmic responses occur, as a rule, only within a restricted range of strength of stimulation. If either stimulus be increased in strength, the response during the compounding of the two stimuli ceases to be rhythmic.

7. As already published, a rhythmic response is obtainable on rapid division of the spinal cord. The movements of this are exactly similar to the movements of progression. There is first a phase of maintained flexion, and at the end a phase of maintained extension. These, on the whole, are arrhythmic, and between them is a phase of " balance " in which the rhythmic movements are most perfect.

8. This previously led to the suggestion that the rhythmic phenomenon is conditioned by a balance of two equal and opposite activities, that is to say, by activities which produce in the same centre equal and opposite effects (excitation and inhibition). In the experiments here given there seems to be evidence that this suggestion is correct.

9. The various rhythmic movements—during stimulation, rhythmic rebound, on compounding equal and opposite stimuli, on rapid division of the spinal cord, on unipolar electric stimulation of the cut surface of the

spinal cord, and the rhythmic movements which occur in narcosis—are closely similar, and seem to be instances of the rhythmic phenomenon of progression.

10. It occasionally happens that a rhythmic response which more resembles the scratch-reflex is obtained on simple peripheral stimulation. There both antagonistic muscles contract at the same time, and there occur reciprocally alternate "beats" as depressions from the plateaus of maintained contraction.

11. The explanation of the occurrence of rhythmic action seems best to be given on the assumption that the two antagonistic centres mutually inhibit each other, and that they are very nearly equally activated by the evoking stimuli. In such a case there will be balanced against each other in each centre antagonistic forces of excitation and inhibition. And in this balance and a phenomenon of fatigue and recuperation of the mutual inhibition is probably to be found the explanation of rhythmic activity.

12. The occurrence in the simple reflex of "rebound contraction after excitation," described previously, and of the allied "rebound relaxation after inhibition," here described for the first time, seems to shew that even there there may be a double excitation of the two antagonistic centres.

13. Evidence given here and in other cases seems to cast doubt upon certain hypotheses of the nature of rhythmic activity :—

(*a*) As the phenomenon of relaxation not infrequently occurs in time before the accompanying phenomenon of contraction in the antagonist it seems hardly possible that the "drainage" theory of inhibition is an adequate one, or that it can serve as a basis for a theory of rhythmic activity.

(*b*) That the phenomenon is not conditioned intrinsically by the conditions of metabolic activity in the moto-neurones is shewn by the experiments here given, in which two stimuli, when given separately, condition arrhythmic responses, but when compounded in appropriate strengths condition rhythmic responses. This can only mean that the centres normally discharge arrhythmically—as regards such coarse rhythms as that of progression—and therefore that the rhythm is conditioned by some property of their interdependence and inter-relationship.

(*c*) On the other hand, the present experiments demonstrate—as the author has done previously—that the rhythm is conditioned centrally and not by an "automatic" peripheral proprioceptive interference. In addition to this the present experiments show that the rhythm on compounding of opposite stimuli may be obtained in the low spinal preparation. This shows that the rhythm is conditioned at the lowest levels, and is not produced, for instance, by the evocation of the activity of a rhythmically discharging centre in the higher parts of the nervous system—a possibility that could not be overlooked if the phenomenon occurred alone in the decerebrate preparation.

14. A possible explanation of the conditioning of rhythmic activity is given in the present paper in a contracted form. It has already been presented, along with a more detailed account of these experiments, to the University of Edinburgh.

Reflex Rhythm Induced by Concurrent Excitation and Inhibition.

By Alexander Forbes, M.A., M.D., Harvard.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received April 11,—Read May 16, 1912.)

(From the Laboratories of Physiology in the Harvard Medical School and in the University of Liverpool.)

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I. *Introduction.*

In a paper on "Antagonism between Reflex Inhibition and Reflex Excitation,"[*] Sherrington has shown that the combined effects of excitatory and inhibitory stimuli produce a response intermediate between those of the two stimuli acting singly. Prof. Cannon, of Harvard, called my attention to certain minute oscillations which occur in Sherrington's myograph line where it represents the response of muscle to the combined effects of the opposed stimuli,[†] and suggested that these might have an important significance. They might mean that the nerve impulses in breaking through the opposition imposed by inhibition were subjected to a condition analogous to a stream of air passing out from a tube under water.[‡] It seemed possible that just as the air bubbles will be larger and more infrequent if the opening of the tube be deeply submerged than if near the surface, so the nerve impulses might be rendered more intense and more infrequent in overcoming the opposing influence of inhibition.

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[*] 'Roy. Soc. Proc.,' B, vol. 80, p. 565.

[†] *Cf.* also 'Roy. Soc. Proc.,' B, vol. 81, p. 258.

[‡] *Cf.* Rosenthal, 'Die Atembewegungen und ihre Beziehungen zum Nervus Vagus,' Berlin, 1862.

On account of the impossibility of recording nerve impulses with the myograph I have planned to investigate the question by electrical methods. But in the course of another series of experiments I have obtained records whose bearing on the problem renders them of interest.

II. *Method.*

In the experiments in which these records were obtained the method was that employed throughout the experiments already reported.* Briefly, it was as follows:—The animal (cat) was decerebrated under ether (or chloroform-ether) anæsthesia. All or nearly all muscles were paralysed by section of their nerves, except the right vastocrureus (knee extensor). In some cases the right peroneal nerve was stimulated to produce reflex inhibition and the left popliteal nerve to produce reflex excitation; in some cases the whole left sciatic nerve was used for excitation, and either the whole right sciatic nerve or the right popliteal or peroneal was used for inhibition. The femur was fixed, and the apparatus so arranged that contraction of the vastocrureus muscle was shown by a rise in the myograph line.

The afferent nerves were stimulated by faradisation from two induction coils. The primary current, common to both, was interrupted between 30 and 50 times a second by a circuit breaker run by a motor. In the later experiments (at Liverpool) a commutator was introduced into the secondary circuit used for inhibitory stimuli, so arranged that it delivered to the nerve only break shocks and those alternately in ascending and descending direction. Defects which resulted from wear of the commutator after many experiments appeared to have an important effect on the reflex responses which will be discussed later.

The strength of the stimuli was estimated in accordance with Martin's scale.† In the earlier experiments (at Harvard), coils calibrated by him were employed. In the later experiments (at Liverpool), an empirical approximation to Martin's units was used.

III. *Observations.*

Two types of observation are dealt with here; those in which two afferent nerves were simultaneously stimulated, and those in which only one nerve was stimulated.

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* "Reflex Inhibition of Skeletal Muscle," in course of publication in 'Quart. Journ. Exp. Physiol.'

† 'Amer. Journ. Physiol.,' vol. 22, pp. 61 and 116.

(a) *Oscillations Resulting from two Opposed Stimuli.*

In the case of the simultaneous stimulation of the two afferent nerves, excitatory and inhibitory, various relative strengths of the opposed stimuli were employed. In an early experiment, the inhibitory stimulus being strong enough almost to prevent contraction, slight oscillations were produced by combined stimulation, as is shown in fig. 1.* The figure shows first the contraction from the excitatory stimulus acting alone, then the response resulting from an excitatory stimulus of the same strength opposed by inhibition two minutes later. The oscillations in this response occur at a rate of about seven a second.

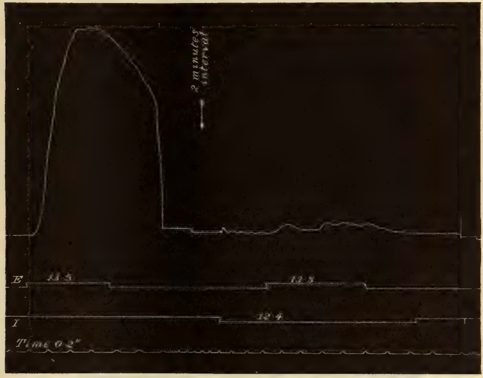

Fig. 1.—Vastocrureus preparation. Time-marker below records intervals of $\frac{1}{5}$ second. (Harvard.)

In another animal with marked decerebrate rigidity, the opposed stimuli were carefully adjusted until, when applied simultaneously, no immediate change in the length of the muscle occurred. Almost immediately,

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* In this and all subsequent figures, ascent of the myograph line means contraction of the muscle. The excitatory stimulus is marked by a *rise* in the upper signal line (E). The inhibitory stimulus is marked by a *fall* in the lower signal line (I). The strength of stimulus in Martin units is indicated by figures on the records. All read from left to right.

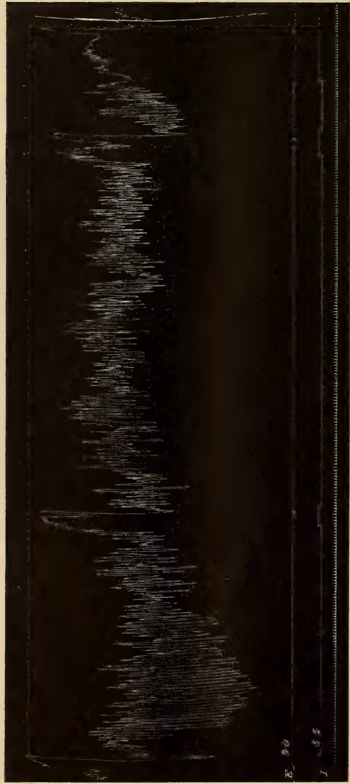

Fig. 2.—Time-marker records in seconds. (Harvard.)

however, marked irregular oscillations appeared and continued as long as the combined stimulation was maintained.

In a third animal in which decerebrate rigidity was not marked, the inhibitory stimulus was made strong enough to reduce the contraction of the muscle to about half the amount produced by the excitatory stimulus alone. The oscillations, which were even more marked than in the case described above, are shown in fig. 2. The excitatory arc was stimulated with 9 units, the inhibitory arc with 5·5 units. Here it will be seen that the muscle was in a state of tremor when the excitatory stimulus was first applied, but the resulting contraction was comparatively free from tremor; then, when the inhibitory stimulus was added, the resulting relaxation was immediately followed by irregular oscillations at a rate of four or five a second. With continuance of the combined stimulation these became more marked and more regular, and slowed to a rate of about one a second.

In a more recent experiment at Liverpool, similar but less marked oscillations were obtained by simultaneous opposed stimuli (fig. 3). Here the inhibitory stimulus was applied to the whole sciatic nerve. The electrodes had been in contact with the nerve for a long time, and local impairment had occurred. The strong stimuli indicated in the figure do not, therefore, represent correspondingly strong central stimuli. The oscillations occur at a rate of about eight or ten a second.

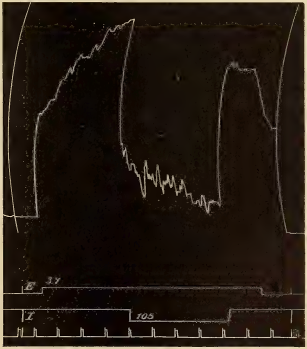

Fig. 3.—Time-marker records in seconds. (Liverpool.)

(b) *Oscillations Resulting from a Single Stimulus.*

In several cases I have observed oscillations during the application of an inhibitory (ipsilateral) stimulus alone. The significance of these I take to be dependent on the fact reported by Sherrington and Sowton,* that by modifying the character of electrical stimulus applied to an ipsilateral afferent nerve, the usual inhibitory reflex effect in the extensor muscle may be replaced by excitation. They find that weak or inabrupt stimuli tend to produce the excitatory effect, while stronger and more abrupt stimuli inhibit. Evidently, the stimulus applied to the ipsilateral nerve exerts both an inhibitory and an excitatory influence on the extensor motoneurones, and it depends on the character of the stimulus, perhaps also on the condition of the preparation, which influence dominates.

The authors propose an explanation of their findings, on the assumption that there are two sets of afferent nerve fibres responding in different degrees to different kinds of stimuli. I have already proposed an alternative explanation,† namely, that there is only one set of afferent fibres, but that they branch on reaching the cord, and are connected with two centres of opposite influences. In support of this is the well-known fact that most afferent fibres do branch on reaching the cord, also the fact that there are known to be two centres of opposite influence upon extensor muscles. There is a local centre in the spinal cord through which an afferent nerve may inhibit the extensors, and there is a centre in the region between the hind edge of the pons and the corpora quadrigemina which is essential to the maintenance of decerebrate rigidity, and is thus shown to exert an excitatory influence upon the extensor.

Altogether, four possible explanations may be framed for the presence of the opposed influences resulting from the single stimulus, as follows:—

A. A single set of fibres acts upon a single centre, but exerts a different influence upon that centre under one kind of stimulus from what it does under another kind.

B. A single set of fibres branches, and acts on two reflex centres of opposite influences.

C. Two sets of afferent fibres from qualitatively different receptors, and themselves differently sensitive, act with opposite effects upon a single centre.

D. Two sets of fibres, of qualitatively different sensitivity, act on two centres of opposite influence, and each set of fibres passes to its proper centre.

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* 'Roy. Soc. Proc.,' B, vol. 83, p. 435.

† *Loc. cit.*

Of these four possible explanations, I believe B involves less needless assumptions than any of the others. On this assumption a reflex view of the knee-jerk would call for a third reflex arc, one of excitatory influence whose centre must be local, since it clearly does not involve the hind-brain directly.

In this connection it is of interest to consider a record obtained from the knee extensor with a series of stimuli of progressively increasing strength delivered to the ipsilateral sciatic nerve (fig. 4). The record is in certain respects similar to those shown by Sherrington and Sowton.* It presents one difference: with a stimulus of 18 units three phases occur in response to a single stimulus—initial contraction on application of the stimulus, inhibition during the continuance of the stimulus, and "rebound contraction" on cessation of the stimulus.† Sherrington and Sowton record the first two phases with weak stimuli and the last two phases with strong stimuli, but in no case more than two phases with any one stimulus.

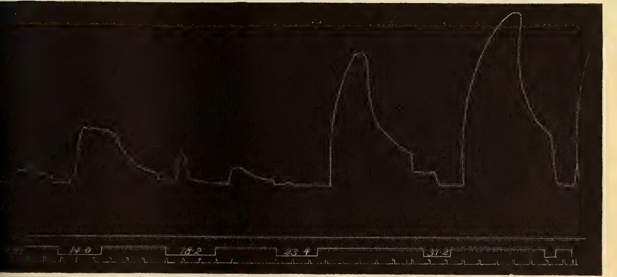

Fig. 4.—Time-marker records in seconds. (Liverpool.)

They have elsewhere‡ proposed tentatively an explanation of "rebound contraction" as the delayed expression of the excitatory content in the ipsilateral stimulus. On the same lines it seems to me that the three phases occurring in response to the single stimulus of 18 units (fig. 4) may possibly be explained as the discharges of the three reflex arcs assumed above on the view that the knee-jerk is a true reflex. The initial contraction would then be due to the arc involved in the knee-jerk with its short latent period. The inhibition following it, and persisting throughout the application of the stimulus, would be due to the preponderant influence of the spinal inhibitory centre. The "rebound contraction," on cessation of the stimulus, would be due to the deferred action of the excitatory centre in the hind-brain or mid-brain. Such an interpretation is only a tentative conjecture at best, but it is quite clear that there is an excitatory content as well as inhibitory in the ipsilateral stimulus.

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* *Loc. cit.*

† *Cf.* Graham Brown, 'Quart. Journ. Exp. Physiol.,' vol. 4, p. 366.

‡ Unpublished paper on 'Reflex Rebound,' 1911.

I have referred above to the defect in the commutator which appeared to influence reflex response. I was unable to trace the cause of the defect or to learn its exact nature. It was most clearly revealed by failure to maintain an even tetanus in the nerve muscle preparation. The influence which it appeared to have on reflex response was the relative accentuation of the excitatory content of the ipsilateral stimulus. This effect was inferred from the following facts. In three consecutive preparations with which the commutator was employed for inhibitory stimuli after months of regular use, the threshold of inhibition was found to be abnormally high. That is, it required a stimulus so strong that it threw the muscles of the trunk and fore-limbs into marked activity, and several times as strong as is usually needed to produce effective inhibition. In two of these preparations, after prolonged inhibitory stimulation, the subsequent inhibitory response was improved instead of being impaired as is usually the case.*

These facts seem to indicate that the quality of the stimulus delivered by the commutator was such as to favour the excitatory content in the reflex effect, and thus partly mask the inhibitory content. Possibly the reflex condition of some of these animals favoured the appearance of the excitatory content.

In one of the three preparations just described, a stimulus of 32 units applied to the ipsilateral sciatic nerve only just sufficed to produce inhibition of the extensor. Stimulation of the sciatic nerve with 42 units produced the oscillatory response shown in fig. 5 A. In a preparation which showed effective inhibition with 7 units applied to the ipsilateral peroneal nerve, a stimulus of 5 units also applied to the peroneal nerve caused similar but less marked oscillations (fig. 5 B).

It seems to me probable that these oscillations occurring upon stimulation of a single ipsilateral nerve are akin to those which result from the stimulation of two afferent nerves whose effects are opposed, and that they are due to the opposition of reflex influences arising from the single stimulus. At all events, they seem to be most evident when the excitatory content of the ipsilateral stimulus is demonstrable

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* Forbes, 'Reflex Inhibition,' etc., *loc. cit.*

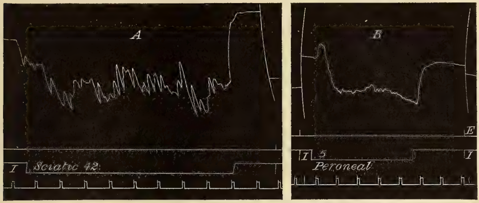

Fig. 5.—Time-marker records in seconds. (Liverpool.)

IV. *Conclusion.*

Graham Brown has reported,* in the "de-afferented" low spinal preparation, regular movements of progression (*i.e.*, alternate contraction and relaxation in both extensors and flexors) occurring in a transition from a predominantly flexed state to a predominantly extended state. He regards this as a feature of "neural balance," and concludes† that rhythmic activity is characteristic of concurrent flexor and extensor influences. Although, in the cases I have described, the oscillations are usually far more rapid, and never attain the regularity and orderly character of the walking reflex or the scratch reflex studied by Graham Brown, yet the analogy is striking and probably highly significant. Perhaps all are manifestations of a general tendency of opposed influences in reflex centres, although themselves continuous, to produce rhythmic activity. If so, it is conceivable that some conditions of intensity or time relations produce the regular movements of progression by enabling the centres to fall into a rhythm natural to them, whereas other conditions entering into my experiments just miss the natural rhythm of the centres and produce a confused rhythm instead.

The general conditions underlying the production of rhythmic activity from a constant stimulus or source of energy are of fundamental interest. No general law covers all cases, but the following conditions obtain in a number of instances, notably that of the stream of air emerging from a tube under water. A source of energy tends to produce an increasing force (A) opposing a relatively constant force (B) which tends to keep the energy pent up or potential; when (A) becomes greater than (B) the accumulated energy is released and becomes kinetic. The force (B) need not be constant, but if it increases during the increase of (A), its rate of increase must fall off below the rate of increase of (A) before the release can occur. The crucial point is this: that when once the release of energy begins it proceeds until more energy is released than is represented by the excess of (A) over (B). The condition which determines this may be figured as a sort of momentum of discharge, although it may not involve inertia in any strict sense of the word.

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* 'Roy. Soc. Proc.,' B, vol. 84, p. 308.

† 'Quart. Journ. Exp. Physiol.,' vol. 4, pp. 393—394.

I believe this consideration is important in connection with vital rhythmic activities. Verworn* has described the series of events which condition intermittent strychnine convulsions. He states that with a violent discharge there is a fall of irritability to the zero point. But he does not emphasise the particular condition which makes the tissue discharge intermittently instead of continuously, namely, that the discharge once started does not stop when the excess of tension has been relieved, but proceeds to completion. To develop rhythm of discharge there must be an approximation to the "all or none law" of the heart beat. A group of "biogen molecules" (Verworn), combining with oxygen and giving off cleavage products in accordance only with the law of mass action, would yield a continuous response to a continuous stimulus.

I believe these considerations have a general bearing on the oscillations shown in the figures. We may suppose that there is in any given case a degree of activity which would be the resultant of the opposed tendencies if equilibrium ever occurred. If these tendencies were in stable equilibrium at this neutral point any oscillations occurring at the outset would diminish and disappear as in the case of a pendulum coming to rest. But the fact that the oscillations generally do not diminish, and sometimes increase, as in fig. 2, shows that at the imaginary neutral point the tendencies are in unstable equilibrium. Some property of the discharge carries it past this neutral point and prevents the establishment of an equilibrium in which the discharge would steadily consume the excitatory tension. There seems to be in the discharge of reflex impulses, and perhaps in all vital activities, some phenomenon akin to the "all or none law" with its refractory period, which makes for intermittence of response.

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* 'Arch. für Anat. u. Physiol.,' 1900, p. 174.

Antelope as a Reservoir for Trypanosoma gambiense.

By H. L. Duke.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received April 20,—Read June 6, 1912.)

In considering the problem of the prolonged infectivity of *Glossina palpalis* along the shore of Lake Victoria Nyanza, the subject must be approached from three points of view:—

Firstly. Some idea of the percentage of infected wild flies must be obtained from time to time, in order to gauge the efficacy of the reservoir.

Secondly. The wild animals frequenting the lake shore must be examined for *T. gambiense.*

Thirdly. The effect of *T. gambiense* on these animals should be studied in the laboratory.

With regard to the requirements (1) and (2), it has been pointed out in a recent report covering the period from March, 1911, to January, 1912, that the percentage of infected flies along the Chagwe coast line is still 0·014 per cent., *i.e.*, a very slight diminution from that reported in June, 1909. During the interim no natives have been allowed in the neighbourhood of the fly, except those in the employ of the laboratory, who are under constant observation.

On Damba Island in May, 1911, Carpenter obtained an infection of a monkey with *T. gambiense*, using 880 wild fly, and two out of four situtunga antelope shot on this island were found to harbour this trypanosome. For Damba Island, therefore, it would appear that the rôle of the antelope in keeping up the infectivity of the wild fly is conclusively established. The conditions on this island are, however, in many ways exceptional, as the fly and antelope are brought into very frequent contact.

Under such conditions it would not be necessary for the antelope to remain infected over a very protracted period of time in order to keep up the infectivity of the fly. In other words, a single infected antelope let loose on Damba Island would soon infect a large number of *Glossinæ*, which would in turn have access to numerous situtunga. Nothing short of a very rapidly acquired immunity to the disease in the antelope could prevent a relatively large number of fly being infective at any given time. On the mainland the opportunities for the fly to bite these animals probably occur much less frequently; and bearing in mind the small percentage of flies capable of carrying *T. gambiense*, it becomes of great importance to know how long an antelope can remain infective.

That these animals may remain infective to fly for a period of at least 10 months after their original infection with *T. gambiense* has already been proved by the study of the antelope in captivity at Mpumu. The animals during this period remain in excellent health, as may be judged from the fact that two young bushbuck have been born in captivity.

The infectivity of these antelope to laboratory-bred *G. palpalis* gradually diminished, until in March, 1911, it appeared probable that the parasites had disappeared from their blood. Up to this date the evidence of infectivity afforded by the presence of trypanosomes in the laboratory-bred *G. palpalis* fed upon the buck, proved sufficiently regular without resorting to blood injection. This latter method was considered a more searching and final test. The experiments given in this paper represent the continuation of these investigations from March, 1910, to January, 1912. During this period laboratory-bred *G. palpalis* were fed upon the antelope, and their blood was also tested by injection into susceptible animals. The injection test, rendering possible the control of considerable quantities of blood, gradually came to supersede the employment of clean flies. There is no reason to doubt that an antelope infective to fly will also give a positive result upon inoculation of its blood into a susceptible animal, and *vice versâ*. The objection to the fly test consists in the small proportion of the laboratory-bred flies used which are capable of harbouring the trypanosome. The expenditure of time and trouble is also obviously far greater where a box of flies is concerned than the mere injection of a few cubic centimetres of blood. In several of the more recent experiments the flies were dissected without ever having fed upon a clean susceptible animal, the presence or absence of flagellates being sufficient evidence as to the infectivity of the antelope.

A few preliminary experiments were undertaken to determine whether the animals had acquired any immunity to *T. gambiense*. These are included below. This question has, however, only been superficially dealt with, as the bushbuck selected as most suitable for the experiment became pregnant.

Reedbuck 2357.

Original infection by laboratory-bred flies containing human strain of *T. gambiense*, April, 1910. Latest evidence, previous to this discussion, of the infectivity of this antelope was obtained in January, 1910, when it proved infective to laboratory-bred *G. palpalis*.

With the exception of Experiment 535, all these experiments were fed upon fowls after their original feeds upon the antelope.

Fly Experiments.

Expt. No.	Period for which flies fed upon 2357.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
47	May 9—20, 1911	29	25	29	0	55	—	
53	May 12—20 „	34	31	33	0	52	—	
207	July 18—25 „	98	87	98	1	48	—	41st day fly. Only gut infected. Tryps. + + +
282	Aug. 10—16 „	91	75	91	0	53	—	
532	Nov. 25—30 „	64	39	64	0	38	—	Never fed on clean monkey.
535	Nov. 29—Dec. 5	89	61	89	0	37	—	Fed upon monkey throughout experiment.
	Totals............	405	318	404	1			

The flagellated fly obtained in Experiment 207 was remarkable in showing negative proventriculus and salivary glands, although dissected on the 41st day of the experiment.

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.	c.c.		
14.12.11	569		6	—	Monkey.
24.1.12.........	630	8		—	„
10.2.12.........	649	10		—	„

The citrated blood mentioned in the above, and also in the ensuing injection experiment, consists of an admixture of the freshly drawn blood with a citrate solution consisting of sodium citrate 1 per cent. and sodium chloride 0·8 per cent. The mixture is arranged so that the blood just loses its power of clotting, the proportion being about three parts of blood to two of citrate. This antelope was thus shown to harbour *T. gambiense* 15 months after its original infection. Since that date, *i.e.*, a period of seven months, no positive evidence has been obtained.

Reedbuck 2431.

Original infection by laboratory-bred flies carrying human strain of *T. gambiense*, May, 1910. Latest evidence, previous to this discussion, of the infectivity of this antelope obtained in November, 1910, when it proved infective to laboratory-bred *G. palpalis.*

Fly Experiments.

Expt. No.	Period for which flies fed upon 2431.	Number of flies. 1st day.	30th day.	Dissected during experiment.	Containing flagellates.	Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
93	May 29—June 5, 1911	54	43	54	0	51	—	
217	July 22—29, 1911 ...	93	89	93	0	46	—	Fed on monkey throughout the experiment.
314	Aug. 21—26, ,, ...	76	55	75	0	51	—	
586	Dec. 26—31, ,, ...	73	42	73	0	31		Never fed upon clean monkey.
590	Dec. 28, 1911—Jan. 1, 1912	73	51	73	0	34		,, ,,
	Totals	369	280	368	0			

Experiments 93 and 314, 586 and 590, were all nourished on fowl's blood during the first 30 or so days of the experiment. In Experiment 93 one fly was found infected with flagellates; these, however, as will be pointed out on another occasion, were derived from the fowl, which was found infected with *T. gallinarum.*

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected. Pure.	Citrated.	Result.	Animal used for injection.
		c.c.			
23.1.12.........	629	6		—	Monkey.
16.2.12.........	656	6		—	,,

Since November, 1910, therefore, during a period of 15 months, Reedbuck 2431 has given no evidence of infectivity to *T. gambiense.* Attempts will be made forthwith to reinfect this antelope first with flies and subsequently by direct blood injection. In the event of a negative result the serum reactions will then be investigated.

Reedbuck 2359.

Original infection April, 1910, with human strain of *T. gambiense.* Latest evidence prior to this paper of the infectivity of this animal obtained in December, 1911, when it infected laboratory-bred *G. palpalis.*

Fly Experiments.

Expt. No.	Period for which flies fed upon 2359.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
76	May 19—27, 1911	67	63	67	0	53	—	
78	May 20—27 „	49	42	49	0	53	—	
548	Dec. 6—11 „	94	59	94	0	40	—	Fed on monkey throughout experiment.
	Totals	210	164	210	0			

Experiments 76 and 78 were nourished on cock's blood throughout the earlier days of the experiment.

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.			
16.5.11.........	62		2½ c.c.	—	Monkey.
16.5.11.........	63		20 ♏	—	White rat.
15.7.11.........	199	5		+	Monkey.
24.1.12.........	631	8		—	„
16.2.12.........	657	7		—	„

In the case of Experiment 199 the incubation period in the monkey was 18 days. A curious point about this monkey, established by my own experiments and those of Miss Robertson, is the relatively large number of positive flies which have been found in boxes fed upon it. Full confirmation is at present lacking, but it would seem that the prolonged sojourn of *T. gambiense* in the buck had resulted in a strain peculiarly adapted to development in *G. palpalis.**

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* *Cf.* Bushbuck 2371, *infra.*

It will be seen that this buck still harboured *T. gambiense* 15 months after its original infection. For the last seven months, however, no positive evidence has been forthcoming. Experiments to try and re-infect this antelope will be now undertaken.

Bushbuck 2371.

Original infection April, 1910. Latest evidence of infectivity obtained in December, 1910, when it infected laboratory-bred *G. palpalis.*

Fly Experiments.

Expt. No.	Period for which flies fed on 2371.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
80	May 22—27, 1911	59	50	57	1	53	+	Monkey died of the disease after 130 days.
220	July 24—29, „	110	76	110	0	48	—	Fed on monkey throughout the experiment.
	Totals	169	126	167	1			

Experiment 80 was nourished on a fowl during the earlier days. The positive fly showed a characteristic infection of salivary glands and gut, while the proboscis was negative. It was dissected on the 53rd day of the experiment and infected the monkey on the 50th day.

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.	c.c.		
31.5.11.........	97		3	—	Monkey.
29.11.11	536	5		—	„
23.1.12.........	628	7½		—	„
10.2.12.........	653	6		+	„

From the above experiment it will be seen that this antelope proved infective with *T. gambiense* 22 months after its original infection with laboratory-bred *G. palpalis.* The incubation period in the monkey was 10 days.

Previous to Experiment 653 a period of nine months had elapsed without a positive result being obtained. Considerable caution is therefore necessary in pronouncing these antelope negative. It may be noted here that, as in the case of Monkey 199 inoculated from Reedbuck 2359, the strain of trypanosomes in Monkey 653 appears peculiarly adapted to development in *G. palpalis*. Sufficient time has, however, not yet elapsed to definitely establish this point.

Waterbuck 2378.

Original infection, April, 1910. Latest evidence of infectivity afforded by positive *G. palpalis* in August, 1910.

Fly Experiments.

Expt. No.	Period for which flies fed upon 2378.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
344	Aug. 30—Sept. 2, 1911	65	45	64	0	42	—	

These flies were nourished on a cock during the earlier days of the experiment.

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.	c.c.		
2.5.11	35		4	—	Monkey.
22.7.11.........	216	3		—	”
23.1.12.........	627		4½	—	”
10.2.12.........	650	8		—	”

There is therefore no evidence to show that the waterbuck has been infective to *T. gambiense* during the last 18 months. The last positive experiment occurred four months only after the original infection. On August 10, 11, 12, 1911, this waterbuck was fed upon by positive flies. Experiment Box 22, which was subsequently shown to contain an infected fly, and which had proved infective to a number of animals. Sufficient experiments have not been carried out to justify a decision as to the immunity of this waterbuck. These will be undertaken shortly. The size of the animal and its resentment of handling make the satisfactory application of the fly test very difficult.

There is, however, every reason to believe that the infected fly of Box 22 must have fed at least once upon this waterbuck, and the animal apparently resisted re-infection. For some time past it has been suffering from an injured foot, which has prevented more extensive investigations being carried out.

Duiker 648.

Originally infected, February, 1911. Latest evidence of infectivity, April, 1911.

Fly Experiments.

Expt. No.	Period for which flies fed upon 648.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
16	April 24—29, 1911	19	16	18	1	96	+	45th day fly.
22	April 25—29 „	25	24	25	1	123	+	122nd day fly.
13	Aug. 3—4 „	46	37	46	0	105	—	Miss Robertson's experiment.
	Totals............	90	77	89	2			

The salivary glands of the positive fly of Experiment 16 were not observed. The fly of September 22 showed a characteristic infection of gut and salivary glands.

Injection Experiment.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for infection.
		Pure.	Citrated.		
6.2.12	637		c.c. $10\frac{1}{2}$	—	Monkey.

Bushbuck 2328.

Originally infected, April, 1910. Latest evidence of infectivity obtained in August, 1910, when this antelope infected laboratory-bred *G. palpalis* with *T. gambiense*.

From June 3 to 10, 1911, 10 months after last evidence of infectivity, this antelope was fed upon daily by Experiment 22. It was desired to ascertain whether any degree of immunity to *T. gambiense* had been acquired, with a view to further experiments with the serum. Bushbuck 2328 has, with the exception of the first few months after infection, always proved negative. As in the case of Waterbuck 2378, the last evidence of infectivity was obtained some four months after infection, the other bushbuck and reedbuck remaining infective for some considerable time longer. It appeared therefore very probable that the trypanosome had died out in this particular antelope.

Fly Experiments.

Expt. No.	Period for which flies fed upon 2328.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
194	July 12—17, 1911	76	65	76	0	50	—	
206	July 18—25 ,,	95	78	95	0	48	—	Fed on monkey throughout the experiment.
	Totals	171	143	171	0			

Experiment 194 was fed upon cock's blood during the earlier part of the experiment.

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.	c.c.		
18.4.11.........	8		1½	—	White rat.
18.4.11.........	9		4½	—	Monkey.
28.6.11.........	176	2½		—	Monkey.
28.6.11.........	177		1	—	White rat.
3.8.11	244	5½		—	Monkey.
6.2.11	638		11½	—	,,

In the above table the line indicates the demarcation between experiments carried out before and after the attempt at re-infection in June, 1911. It will be seen that in no case did infection result. This antelope thus appears to have acquired a certain degree of immunity to *T. gambiense*.

The following Experiment 195 represents an attempt to influence the course of an infection of *T. gambiense* in a monkey by treating the latter with the serum of Bushbuck 2328.

Monkey 195 received injections of the serum of Bushbuck 2328 on four occasions as follows :—

On July 12, 1911, monkey received 15 ♏ serum subcutaneously.
„ 22, „ „ „ 30 „ „
On August 2, „ „ „ 2¼ c.c. „ „
„ 15, „ „ „ 2 „ „ „

No symptoms of serum-anaphylaxis were observed.

On August 16 to 17, 1911, the monkey was fed upon by positive Experiment 22. On August 30, *T. gambiense* first appeared in the blood of the monkey, the incubation period being thus 13 to 14 days.

On September 3, 1911, the monkey received 2½ c.c. serum of Bushbuck 2328, without any diminution being observed in the number of trypanosomes.

February, 1912, Monkey 195 is still alive and apparently in good health. The interval, however, is not long enough to warrant any criticism as to the effect of the above treatment on the disease, which would, however, appear to be pursuing quite an ordinary course.

The most striking point about this experiment is the long incubation period of the disease in the monkey. Normally after infection with Box 22, trypanosomes appeared in the monkey's blood after an interval of seven days only. Further experiments with this bushbuck had to be discontinued owing to the animal becoming pregnant.

This bushbuck has apparently successfully resisted the attempt to re-infect it with positive flies in June, 1911. Further investigations are essential before a definite opinion can be pronounced as to its immunity. Experiments will be carried out with a view to re-infection by direct blood injection, and following a negative result, the reaction of the serum will be investigated.

The following experiments were undertaken to ascertain whether the serum of these antelope had acquired any immunising properties.

The animal selected was Reedbuck 2359, which at the time, June, 1911, had shown no evidence of infectivity for some seven months. As may be seen above, this animal was subsequently proved to be infective in July, 1911—one month after the experiments were carried out.

(1) Has the serum of Reedbuck 2359 any trypanolytic action on *T. gambiense ?*

Two tubes A and B were prepared, each containing $\frac{1}{3}$ vol. serum and $\frac{2}{3}$ vol. citrated *T. gambiense*-blood. Tube A contained serum of Reedbuck 2359; tube B serum of a bushbuck which had never been infected with *T. gambiense.* The tubes were placed for four hours in incubator at 28° C. The contents were examined at intervals during this time, but no difference was observed in the motility of the trypanosomes, and no agglutination was seen in either tube.

(2) Has serum of Reedbuck 2359 any protective action against *T. gambiense?*

(*a*) On June 15, 1911, a white rat received 15 ♏ serum of reedbuck, and on June 16, 1911, 24 hours after, was injected with a small dose of *T. gambiense.* The rat died in 25 days.

(*b*) In two tubes A and B were placed respectively $\frac{1}{3}$ vol. of serum of reedbuck and $\frac{2}{3}$ vol. of citrated *T. gambiense*-blood, and $\frac{1}{3}$ vol. serum of normal bushbuck and $\frac{2}{3}$ vol. of citrated *T. gambiense*-blood. The tubes were kept at room temperature in the dark. The contents of the tubes were examined at intervals of 3, 5½, and 6½ hours, without any differences being noted; 6½ hours after the commencement of the experiment, 6 ♏ of each tube-contents was inoculated into white rats C and D. These rats died in 23 and 21 days respectively.

The *T. gambiense* employed in the above experiments was a strain derived from wild Lake-shore flies. It is to be regretted that no control injection of trypanosomes alone was performed, owing to scarcity of rats and my failure at the time to realise the importance of the point.

It will be noticed that the disease in both cases was extraordinarily rapid. Whether this was due to the trypanosome strain, or to the antelope serum, or to both these factors, cannot at present be decided. The monkey from which the trypanosome was taken was killed two months after the infection. In a subsequent experiment, in which a white rat was injected with another strain of Lake-shore trypanosomes derived from fly, the disease lasted 54 days. The various *T. gambiense* strains at Mpumu all give rise to a fairly protracted disease in rats.

The above facts warrant an enquiry as to the identity of the trypanosomes obtained in monkeys by feeding wild Lake-shore *G. palpalis* upon these animals. The trypanosomes so obtained have always been described by Bruce as *T. gambiense,* though I am unaware of the nature of the evidence adduced in support of this assertion. It is, of course, extremely probable, considering the recent epidemic along the Lake-shore and the nature of local cattle diseases, that these trypanosomes found in wild flies and pathogenic to monkeys are *T. gambiense.* Nevertheless, the question cannot be summarily dismissed, owing to the fact that other species of trypanosomes can produce similar symptoms in these animals. The point of practical importance to be established is whether these trypanosomes are pathogenic to man, and this is obviously very difficult to prove.

In a recent paper I have discussed the differential diagnosis of trypanosomes recovered from the Lake-shore and *T. brucei.* The recent discovery that *T. rhodesiense* is susceptible to the action of human serum is of great interest in this connection, since on theoretical grounds alone a trypanosome which succumbed to human serum *in vitro* and in laboratory animals might well be judged non-pathogenic to man. In the present instance it must be borne in mind that, although these Lake-shore trypanosomes, whether found in wild flies or in wild antelope, may have been originally derived from human beings during the recent epidemic, it is possible that the prolonged sojourn in other hosts may have materially modified the reactions of the original "Uganda" strain.

Apart from its pathogenicity to man, there is little in the behaviour of *T. rhodesiense* to suggest close affinity to *T. gambiense.* Its recently discovered susceptibility to the action of human serum, together with many of its animal reactions, suggest rather resemblances to *T. brucei.* This latter trypanosome has up to the present time been generally considered to be non-pathogenic to man. The mere fact, however, that *T. rhodesiense* was originally recovered from man is sufficient to account for the controversy which has arisen over the attempt to separate this trypanosome from *T. gambiense.*

In the same way with these Lake-shore trypanosomes. Whatever be the scale of virulence established, the final diagnosis will always depend upon the behaviour of the organism in the human subject.

In reviewing the above experiments it will be seen that these antelope are apparently very slowly losing their infectivity for *T. gambiense.* The animals are all in excellent health, and in two out of four cases the most recent evidence has been afforded by positive laboratory-bred *G. palpalis.* It would thus seem that the adaptation of *T. gambiense* in these antelope approaches very nearly the biological ideal, *i.e.*, a compromise between the trypanosome and its host, whereby, without injuring the latter, the parasite becomes peculiarly well equipped to survive in the insect carrier.

Time alone can show whether this balance between parasite and antelope host can be maintained indefinitely. Judging from the case of Bushbuck 2371 it is quite reasonable to expect evidence of infectivity to crop up occasionally at long intervals in these laboratory animals. Surrounded by wild flies in nature such an antelope as 2371 might well prove a far greater danger than would appear from the long intervals occurring between positive experiments in the laboratory. It is, of course, possible that the trypanosomes may be slowly dying out in the buck, and that a greater or less immunity may be left behind. But even if this be so, an animal which can remain infective for 22 months may be looked upon as a true reservoir for the virus of sleeping-sickness. It has only to remain in the neighbourhood of the fly to perpetuate the disease indefinitely among susceptible Lake-shore animals.

The behaviour of the positive flies obtained from Antelope 2371 and 2357, 13 and 15 months respectively after the original infection, is interesting. In the first case, the positive fly did not infect the monkey until the 50th day of the experiment; and in the second instance, a 41-day fly showed no flagellates in either proventriculus or salivary glands. In all previous experiments with these antelope the flies became infective about the 30th day of the experiment.

An explanation of this curiously slow development of the flagellates in the fly may be that the trypanosomes are present in very small numbers in the peripheral blood of the buck. A fly would then obtain an extremely small number of trypanosomes at a feed, whereas formerly a considerable number may have been imbibed.

Some support is lent to this suggestion by the prolonged incubation period in Experiment 199 (Reedbuck 2359), where 18 days elapsed before *T. gambiense* appeared in the monkey's blood.

Conclusions.

1. That the antelope may remain capable of infecting *G. palpalis* with *T. gambiense* for a period of at least 22 months after their original infection with this trypanosome.

2. That there is some evidence to show that an antelope which has ceased to be infective for *T. gambiense* acquires some degree of immunity against re-infection.

The Relation between Secretory and Capillary Pressure. I.—The Salivary Secretion.

By Leonard Hill, F.R.S., and Martin Flack (Eliza Ann Alston Research Scholar).

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(From the Physiological Laboratory of the London Hospital Medical College, London Hospital Research Fund.)

Since Ludwig made the discovery that the secretory pressure of a gland may double that of the arterial pressure when the outflow of saliva is obstructed, no one, so far as we know, has investigated the circulatory conditions in the gland under these circumstances. This has been the object of the present research.* Our method is as follows:—We place a cannula in the duct of the submaxillary gland of the cat or dog and prepare the chorda tympani nerve for excitation. A second cannula is placed in the carotid artery of the opposite side of the neck. Each cannula is connected, either with a mercurial manometer or, as in our latest experiments, with two Leonard Hill pocket sphygmometer gauges. This gauge consists of a thick-walled glass tube with a fine capillary lumen closed at one end where the lumen expands into a small air chamber. Half an inch from the open end there is a side hole. On placing this end in a solution of potash a fluid meniscus rises to the side hole, which marks the zero of the instrument. (Potash is used to keep the tube free from grease.) One end of a piece of rubber is slipped over the open end of the gauge so as to cover the side hole, and the other end then connected with the cannula. The pressure of the saliva or blood forces the meniscus up the gauge, which is graduated in millimetres of mercury and acts as a spring manometer. We find these gauges very convenient to use as they can be placed side by side and the readings compared at a glance. Before making the connections with the gauges we expose the veins which course over the submaxillary gland and contribute to the formation of the external jugular vein. Having found the vein which issues from the gland we tie all the other veins, leaving this one free so that at the right moment we can clip the external jugular and open it so as to observe the outflow of blood from the gland. When all is thus prepared we excite the chorda tympani nerve. As soon as the secretory pressure rises above the arterial pressure we open the vein and observe the flow of blood. We find that under

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* Towards the expenses of this research a grant was made by the British Medical Association.

Table I.

	Salivary pressure.	Arterial pressure.	Venous outflow.
Dog. Morphine, chloroform	140	88	Blood flowing.
„ „ „	190	98	„
„ „ „	164	80	„
„ „ „	157	93	„
Cat. A.C.E.	125	110	„
„ Ether	179	140	„
	160	125	„
	170	125	„
„ „	130	110	„
„ „	160	135	„
„ „	135	110	„
Dog. Morphine, ether	240	130	„

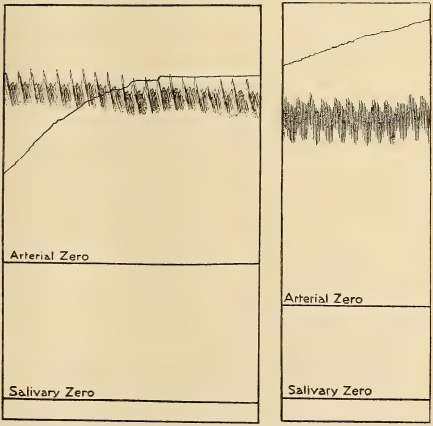

Fig. 1.—Tracings from two different dogs, anæsthetised by morphine and chloroform, showing the arterial (carotid) pressures and the secretory pressures of saliva during excitation of the chorda tympani nerve.

these conditions the blood continues to flow and issues from the vein in a fairly ample stream of a colour more arterial than venous. The gland itself feels tense to the touch. By squeezing the gland we find that we can further raise the pressure of the secretion, while at the same time we impede the outflow of blood. On allowing the secretory pressure to fall to atmospheric pressure we find that the outflow from the vein becomes much ampler; in one experiment it was approximately doubled, *e.g.* 40 drops in 15 seconds as compared with 27 drops in 20 seconds at the highest secretory pressure (240 mm. Hg.). In some experiments we observed the venous outflow from the time we began to excite the chorda tympani. Under these circumstances we find that the outflow from the gland at rest is very slow and the blood venous in colour. On stimulating the chorda, the outflow becomes very ample, the blood arterial in colour, and remains so during the rise of secretory pressure until this pressure rises higher than the arterial, when the outflow becomes lessened and the blood less arterial in colour.

Our results show clearly that there exists in the salivary gland a structural mechanism which protects the capillaries from the maximum secretory pressure which may exist within the alveoli and ducts of the gland. The gland is of the compound racemose type, and composed of alveoli around which a dense network of capillaries is spun. The alveolar cells, 2–3 μ in diameter, are formed of bioplasm, which probably contains some 80 per cent. of water. The wall of the capillaries is formed of a single layer of exceedingly thin cells, less than 1 μ in thickness. It does not seem possible to us that either the alveolar or the capillary cells can support any difference of fluid pressure. The blood, tissue, lymph and cell protoplasm must all be at one and the same fluid pressure, unless there exists some rigid supporting structure, which surrounds the alveoli and limits their expansion. On seeking for histological evidence, we find that Kölliker* and other histologists describe the alveoli as surrounded by a thin structureless membrane, the membrana propria. This can be easily demonstrated in glands which have been treated with strong potash. The membrana propria is thus shown to be a structureless membrane less than 1 μ thick, and wonderfully strong. Kölliker states that, upon injecting the ducts of the glands under high pressures, the secretory cells may be destroyed or separated from the membrana propria, but that this membrane seldom becomes torn. Before this happens, extravasations will take place through the walls of the ductules. We ourselves have seen such extravasations in glands which have been kept secreting for a considerable time at a high pressure. The so-called basket cells also surround the gland cells, there anastomosing by their branches to form a kind of protoplasmic framework, which contains the secreting cells. It has been suggested that the basket cells are contractile. The membrana propria clearly acts to the secreting cells as does the sarcolemma to the muscle fibre. The cells stimulated to secretory activity imbibe water from the capillaries and extrude the salivary secretion into the ductules at a pressure which may double that of the arterial pressure. Meanwhile the capillaries are protected from occlusion by the membranæ propriæ of the alveoli, which, acting like the leather case of a football or the pericardium of the heart, limit the expansion of the alveolar cells. When the secretion is obstructed, the whole gland becomes tense, the veins are diminished in volume, the circulatory pressure in them is raised until the vessels, arteries, capillaries, and veins approximate to a rigid system at arterial pressure, with a fast rate of flow. As the alveoli swell, this favourable condition of the circulation is at first established; finally, however, the vessels may be so far narrowed that the flow becomes lessened, but not stopped, owing to the restraining action of the membranæ propriæ. It seems to us probable that the slackening of the circulation may be due to the leakage of saliva into the intra-alveolar connective tissue. If we cease to excite the chorda, the secretory pressure drops slowly, owing to such leakage. The basket cells may possibly, by their contractile power, help to squeeze the saliva into the ducts. We have no evidence to offer as to such a function. No doubt the membranæ propriæ may be supported by the strands of connective tissue which surround and knit together the alveoli.

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* Kölliker, 'Handb. der Gewebelehre des Menschen,' Leipzig, 1899, vol. 3, p. 46.

We believe that we are here dealing with a fundamental principle in the construction of many parts of the body. The cells in the secreting glands are enclosed on one side by a cuticular membrane which permits their imbibition of fluid but checks their power to swell, thus enabling them to do work and at the same time receive an ample supply of blood. The mechanism reminds us of Pfeiffer's semi-permeable membrane, with this difference, that the living membrane can vary its permeability. The muscle fibres are enclosed by a sarcolemma, the nerve-fibres by a neurilemma, the secreting alveoli by a membrana propria. In each case the membrane may limit the swelling of the protoplasmic content, and so permit a high imbibition pressure within. Turning to the kidney, we find a similar membrana propria enclosing the secreting cells, both of the capsules and of the tubules. The secretory pressure in the obstructed ureter, however, does not rise so high as the arterial pressure. Starling gives the following readings :—*

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* Starling, 'The Fluids of the Body,' 1909, p. 110. Constable, London.

Carotid artery.	Obstructed ureter.
mm. Hg.	mm. Hg.
140	72
138	92
133	88

The structural mechanisms visible in the kidney seem to us altogether opposed to the doctrine that fluid is filtered from the blood into the capsule by the force of the blood-pressure. There are membranæ propriæ which confine the tubules, but no rigid structures which can sustain the capillary wall and convert it into a membrane capable of sustaining a difference of hydrostatic pressure. The glomerulus is a lobulated structure the lobules of which hang in the capsule, just as the ciliary processes hang in a bath of aqueous within the posterior chamber of the eye. We believe that the passage of fluids is determined in both cases by a secretory force acting from the cells which line the vascular processes. The pressure of the fluid within the capsule and of the blood within the glomerular vessels must be the same. In the case of the brain the choroidal fringes secrete the cerebro-spinal fluid; the venous pressure and that of this fluid correspond and are always one and the same. The pressure of the brain against the wall of the skull is also the same; the slightest increase of pressure exerted on the brain squeezes the blood out of the capillaries. If the pressure be made to rise in the cerebral veins, that of the cerebro-spinal fluid rises to the same extent. Suppose fluid be injected, or in some inflammatory state cerebro-spinal fluid be secreted at a higher pressure, then the venous pressure rises concomitantly, since the veins are narrowed by the injected or secreted fluid. We shall show in a subsequent paper that in the eye the same conditions hold good. The ciliary processes are held to secrete the aqueous, and the circulatory pressure in the veins within the eyeball is adjusted to the secretory pressure of the aqueous. The aqueous takes up so much of the total volume and the veins are narrowed to such an extent that the intra-ocular pressure is raised to a considerable figure, some 30–40 mm. Hg. The pressure, both intra-ocular and intra-cranial, depends on the circulation, and ceases with the circulation, but its height is regulated by the secretory action of the cells. In the eye and brain we have the delicate secreting fringes lying in a bath of the secreted fluid, and there exists no supporting structure which can bear off any part of the pressure. We cannot imagine that the fringes are held open as it were by wire springs. The pressure of the secretion outside and of the blood within the capillaries and veins must be the same. In the case of the brain, the cerebro-spinal pressure, the cerebro-capillary pressure, and the cerebro-venous pressure are one and the same, a fundamental fact which has been proved.* In the secreting glands, confined by a capsule, the same conditions must hold good. The capillary, venous, and tissue fluid pressures must be the same, adjusted one to the other, excepting where, as in the salivary gland, membranæ propriæ are drawn taut by the height of the secretory pressure when the duct is obstructed. In the case of the salivary gland, the capacity to produce so high a pressure may be correlated with the need which arises, under certain circumstances, for a very rapid secretion of saliva, *e.g.* to wash out an irritant. The same high secretory pressure is produced by the sweat glands. A rapid secretion of sweat may be required to prevent heatstroke. Here, again, the membranæ propriæ play the same part as in the salivary glands, the secretory force possibly being increased by the layer outside the secreting cells, which is supposed to be contractile. Both the flow of sweat and of saliva may be temporarily obstructed by pressure applied to the ducts.

In the case of the kidneys a lower secretory pressure suffices. The pelvis and the bladder act as reservoirs, the abdominal wall and the diaphragm regulate the intra-abdominal pressure. The whole kidney, just as the salivary gland, is confined by a capsule which allows a certain expansion and no more. Within the capsule, from time to time, there may be more blood and less kidney substance (including tissue, fluid and secretion), or more kidney substance and less blood. Suppose the arterioles of the kidney dilate, the volume of the blood increases and the whole kidney expands. Suppose, moreover, the renal tubules are inactive, not swollen, and the membranæ propriæ are slackened, then there will be within the capsule less kidney substance and more blood. The whole kidney substance will be at the lowest fluid pressure, namely, that of the veins. The same holds good for the salivary gland. Suppose, on the other hand, the tubules are swollen, actively secreting, with the membranæ propriæ expanded, there will be less room within the capsule for blood, the veins will be narrowed, the renal venous-capillary pressure raised, the vascular system approximated to a rigid system, the velocity of the flow increased. The whole kidney will still be at the lowest fluid pressure, viz. that in the veins, but now it will feel tense, for the venous-capillary pressure, under these conditions, is so much higher. It is clear that the salivary or renal cells, swollen and in an active state of secretion, not only produce a secretory pressure in the tubules, but by narrowing the veins influence the height of the blood pressure within and the rate of blood flow through the capillaries and veins. The quality of the blood influences the secretory activity; this, in its turn, influences the blood flow within the kidney. When the ureter is obstructed and the kidney secretes against pressure, the tubules must swell until the membranæ propriæ are drawn taut. In the case of the salivary gland, the swelling of the alveoli is limited by the membranæ propriæ, so that the blood-vessels remain patent in spite of the higher secretory pressure. In the kidney, however, the secretion ceases before the secretory pressure rises to the arterial pressure. Our next point of enquiry must be to determine whether the cells of the tubules are able to swell to such an extent as to impede the circulation. Further experiments will show us what happens to the flow in the renal vein at the moment when the secretory pressure of the urine reaches its highest point.

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* Leonard Hill, 'The Cerebral Circulation,' 1896. J. and A. Churchill, London.

In states of inflammation, as their osmotic pressure increases, the poisoned tissues swell, the arteries dilate, the blood-pressure rises as the swollen tissues press upon and narrow the veins. The vessels approximate to a rigid system, hence the high tension and the pulsatile throb of the inflamed part. As the tissue pressure rises in the part, so does the circulatory pressure, since the tension depends on the arterial pressure; its height, however, being regulated by the metabolism of the tissues and their imbibition pressure. Finally, stasis is produced in the swollen tissues. The surgeon's knife by cutting, or the hot fomentation by softening, relieve the tension and allow an ample flow of blood to and the escape of immunising plasma in the part. It is a question here whether stasis results from inflammatory changes in the blood or from strangulation of the circulation by the increased imbibition and swelling of the tissues. The tissue cells in the skin are confined by strong frameworks of connective tissue, which limit expansion and act in a similar manner as does the capsule to a gland, the sclerotic to the eye, or the skull wall to the brain.

Summary.

(1) When the pressure of the salivary secretion is raised above the arterial pressure the venous flow of blood from the gland continues.

(2) Under these conditions the gland feels very tense; by squeezing it the secretory pressure is raised but the flow of blood from the vein is stopped.

(3) The view is advanced that the membranæ propriæ (aided possibly by the strands of connective tissue which surround the alveoli) limit the expansion of the alveoli and thus protect the circulation from strangulation by the high secretory pressure. It is suggested that limiting membranes in other parts of the body, *e.g.* the kidney, have a similar function.

(4) By the expansion of the alveoli up to the limitation point set by the

membranæ propriæ the veins are so far narrowed as to raise the pressure in the capillaries and veins; this, coupled with the dilatation of the arteries, gives a greatly increased rate of blood flow. The circulatory conditions which pertain in these encapsulated glands resemble those which pertain in the brain and the skull cavity.

The Effects of Ultra-violet Rays upon the Eye.

(Report of experiments carried out for the Glassworkers' Cataract Committee of the Royal Society.)

By Dr. E. K. Martin.

(Communicated by Sir J. R. Bradford, Sec. R.S. Received February 5,—Read March 14, 1912.)

(From the Research Laboratories, University College Hospital Medical School.)

[Plates 4—7.]

In 1909 Messrs. J. Herbert Parsons and E. E. Henderson commenced some experiments on the action of short wave-length light on the lens and ciliary body, using Uviol glass mercury-vapour tubes and examining the lens and its capsule after exposure. To test for damage to the ciliary body too slight to be appreciable microscopically use was made of an observation of Römer's, that in animals sensitised to the blood of another species, hæmolysins were not transmitted from the blood to the aqueous unless the constitution of the latter were altered by a previous paracentesis or an inflammatory lesion of the iris and ciliary body. Positive results were obtained, but the experiments were not sufficiently extensive to be conclusive. I have, therefore, on behalf of the Committee of the Royal Society on Glassworkers' Cataract, repeated and extended the experiments along lines suggested by Mr. Parsons.

In the attempt to determine the effect of rays of various wave-lengths on the media of the eye, attention has been paid to three possible ætiological factors:—(1) The intensity of the light. (2) The part of the spectrum mainly represented in the source of light. (3) The possibility of the inclusion of electrolytic and mechanical as well as of radiant energy.

1. *Intensity.*—It has been customary among investigators of the subject to express the intensity roughly by naming the source of light, the quantity of energy (usually electrical) consumed in its production, and the distance of

the exposed eye from it. Some observers have also attempted to record it in absolute units by expressing it in terms of the universal photometer.

Whilst this latter method is at first sight more exact, the correlation of observations from various sources would be facilitated by expressing the intensity also approximately in terms of one of its physiological results. As the *rôle* of strong light in the production of corneal opacity is now well recognised, and the degree of opacity varies *pari passu* with the quantity of energy absorbed, valuable indication of the intensity of a given source of light from a physiological point of view is afforded by the resultant corneal disturbance—when the exposures are repeated at intervals throughout a considerable period of time.

2. *The Part of the Spectrum mainly Represented in the Source of Light.*—From the physiological point of view the spectrum may be divided into heat, visible and ultra-violet rays, each of which require separate consideration. Putting aside the first, the investigation of which is not included in the present work, the absorption by the media of the eye must be taken into consideration, since only rays which are absorbed are capable of inducing pathological changes in those media.

3. Ocular lesions following exposure to lightning, short-circuits of high-voltage currents, and discharge of Leyden jars should be omitted from the discussion of the effects of radiant energy, on account of the impossibility of determining the value of the electrolytic and mechanical (concussion) factors.

The present enquiry may be divided into three parts:—

I. The absorption of rays by the media of the eye in the visible and ultra-violet portions of the spectrum.

II. The histological changes resulting from exposure to light consisting largely of such rays.

III. The alteration in the iris and ciliary body produced by such rays.

Young rabbits (*i.e.* three or four months old) have been employed throughout.

I. THE ABSORPTION OF RAYS IN THE MEDIA OF THE EYE IN THE VISIBLE AND ULTRA-VIOLET PORTIONS OF THE SPECTRUM.

The spectrophotographic method of recording the results was used, and the media were mounted in cells with parallel sides of quartz. The lens was dealt with in two ways:—

1. Suspended in normal saline and placed at a distance from the slit greater than its focal length, so that a blurred image of the source of light was thrown on the slit. This was necessary to avoid horizontal lines on the

resulting photograph, and the precautions taken quite excluded the possibility of stray light coming in between the cell and the slit.

2. A thin layer of lens substance was squeezed out flat between the parallel sides of the cell. This was done to eliminate any possible apparent absorption due to refraction in the lens.

Visible.

A preliminary series of spectrophotographs were taken to eliminate absorption within the limits of the visible spectrum.

Source of Light.—The crater of a carbon arc.

Instrument.—A two-prism spectroscope, prisms and lenses being of glass.

Plates.—Commercial "orthochromatic," which regularly showed three faint absorption bands in the green, yellow, and red, representing the absorption of the dye used to sensitise the plates to red light.

Result.—All the media were found to be uniformly permeable to rays between the wave-lengths 660 $\mu\mu$–390 $\mu\mu$. (The visible spectrum extended approximately 760 $\mu\mu$–380 $\mu\mu$) (Plate 4).

Ultra-violet.

For the ultra-violet rays the iron arc was the source, and quartz lenses and prisms were employed throughout. Ordinary plates were used, *i.e.* plates containing no dye and hence giving no absorption bands.

The range of wave-lengths covered in this series is 450 $\mu\mu$ to 230 $\mu\mu$.

Cornea.—The cornea offers no resistance to rays of wave-lengths longer than 295 $\mu\mu$, but all those beyond this limit are completely cut off (Plate 5, B).

Lens.—(*a*) Suspended in normal saline. Rays of wave-lengths less than 350 $\mu\mu$ are absorbed completely. The line is not a sharp one, the absorption commencing about 400 $\mu\mu$ (Plate 5, F).

(*b*) Squeezed to different thicknesses. The absorption varies *pari passu* with the thickness of the layer of the lens substance (Plate 5, C, D, E).

It is clear, therefore, that between wave-lengths 400 $\mu\mu$ and 300 $\mu\mu$ there is a group of rays to which the cornea offers no resistance, but which are absorbed by the lens, and are consequently capable of inducing changes in it.

Vitreous.—The vitreous, in a layer 3/16 inch thick, shows a broad absorption band, extending from 280 $\mu\mu$ to 250 $\mu\mu$, with a maximum at 270 $\mu\mu$. The margins of the band are ill defined (Plate 5, H).

These results agree closely with those of the observers referred to below. The shortest interval between the death of the animal and the taking of the observation was 3 minutes, the longest 11 minutes. Observations were also made on the media 1 hour, 5 hours, and 8 hours after the death of the animal, the results obtained being identical with those from fresh specimens.

The photographs were taken at the Imperial College of Science and Technology, South Kensington, those of the visible spectrum in Sir Wm. Abney's laboratory, the ultra-violet series in the Astrophysical Laboratory, by kind permission of Prof. Fowler, F.R.S., to whom I am indebted for valuable suggestions.

II. Histology.

Normal Capsule.

Method of Handling the Anterior Lens Capsule.—The most satisfactory fixative solution for uniformity of results is Zenker's fluid. Formalin, in solution or vapour, gives excellent fixation of the cells, but renders the homogeneous layer of the capsule too elastic to allow of its being fixed flat on a slide. In addition, the epithelium shows a tendency to adhere to the lens substance rather than to the capsule.

The following method has given the most uniform results:—

The globe is bisected at the equator, and the anterior half fixed in Zenker's fluid for two hours, washed, and adherent vitreous removed. The capsule is then cut through round the equator, and the lens shelled out entire, leaving the anterior capsule attached to the ciliary processes by the suspensory ligament. The latter is then divided, and radial incisions made into the capsule, allowing it to be floated, epithelium uppermost, on to a slide, and blotted flat.

As a stain, Ehrlich's acid hæmatoxylin has been used exclusively, as it gives by far the most constant results. The iron-hæmatoxylin methods give clear pictures, but are somewhat irregular, while the aniline stains have the drawback that they colour the homogeneous layer of the capsule, and so spoil the contrast.

Occurrence of Mitoses.—From the systematic examination of the anterior capsule cells of the rabbit's lens mounted as described, it appears that at birth, and for the first six to eight weeks of life, numerous mitoses occur, scattered uniformly round the equator over a zone equal in breadth to a quarter of the radius of the capsule. Cell division in this zone becomes progressively less active till the third or fourth month, and, in animals over six months, 10 to 15 mitoses only are found at the extreme periphery.

Cell division in the pupillary area is extremely rare at any age, and has been found only once in 10 capsules examined systematically for that purpose. The age of the animal and the topography of the normal capsule must therefore be taken into account in discussing the occurrence of pathological mitoses after exposure to light (figs. 1 and 2).

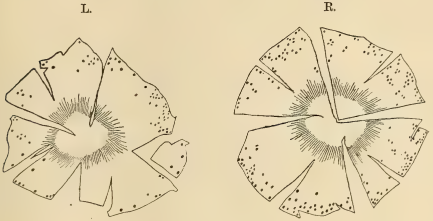

Fig. 1.—Six weeks old.

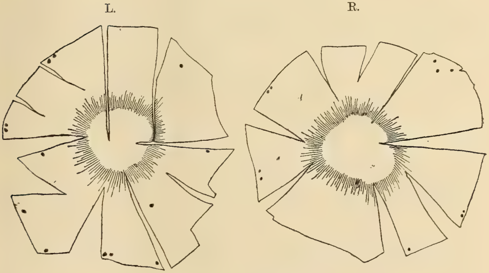

Fig. 2.—Twelve months old.

Anterior lens capsules of normal rabbits. (Dividing nuclei indicated by dots.)

Histological Changes in Lens and Ciliary Body resulting from Exposure to Rays of Short Wave-length.

As a source of light containing the highest possible proportion of short wave-length rays, the Kromayer water-cooled mercury-vapour lamp was employed. This consists of a mercury arc in a quartz vacuum-tube surrounded by a water-jacket with a quartz window.

The photographic spectrum to this lamp extends from wave-lengths 590 $\mu\mu$ to 222 $\mu\mu$, and is most intense between wave-lengths 579 $\mu\mu$ and 254 $\mu\mu$. It emits no heat appreciable by means of a thermometer. The current consumption is 2·5 ampères on a 220-volt circuit.

A. *Single Exposures of High Intensity.*

If the lids are kept apart by a speculum, exposures of one-half to two hours at a distance of 1 inch are followed by the changes in the anterior capsule cells described by Hess (Ref. 10). That is, shortly after the exposure, the cells are swollen and their nuclei show diffuse staining of the chromatin. After 24 hours there is a central area, corresponding to the pupil, in which many of the nuclei are represented by scattered chromatin granules extruded from the still visible nuclear membrane. Surrounding this damaged area is the ring of apparently crowded, deeply stained cells which Hess has described as the epithelial "wall." This "wall" may or may not form a complete circle, and varies in width from about four to eight cells. The appearance is due chiefly to the deeper staining of both nuclei and cell bodies, consequent on the submaximal damage at the pupillary margin, accompanied probably by the heaping up described by Hess. He suggested that the swollen central cells pushed those at the pupillary margin up against the intact ones lying under cover of the iris, and so crowded them closely together.

Two or three days after the exposure, numerous karyokinetic figures are seen in the damaged area, regeneration taking place in the usual way.

The inflammatory changes in the anterior part of the eye correspond with those described in detail by Birch-Hirschfeld (Ref. 7) and other writers as following exposure to the Uviol mercury-vapour lamp, etc. There is intense conjunctivitis with sub-epithelial hæmorrhages, thickening of conjunctival and corneal epithelium, with subjacent round cell infiltration corresponding to the hyperkeratosis and papillary infiltration seen in the skin after exposure to the Kromayer lamp. The pupil is small and the iris hyperæmic, but I have not observed exudates on iris or ciliary body with the exposures given.

That these changes are due wholly or almost entirely to rays beyond the visible violet is shown by the absence of all but a slight conjunctival hyperæmia following exposures of similar duration, in which a benzol cell is interposed between the lamp and the eye. Benzol is transparent to the rays of the visible spectrum but cuts off all those beyond the violet end.

An attempt was made to find some substance permeable to rays of the shorter wave-lengths only. P.-nitro-benzene-azo-dimethyl-aniline in N/10,000 aqueous solution transmits a band in the red and another beyond the violet, but the intensity of the resulting rays is so low as to produce no effect even on the conjunctiva after exposures of 1 hour at 5 inches daily for 2 weeks

B. *Repeated Exposures of Moderate Intensity.*

In this series no speculum was employed and the intensity was below that necessary to cause immediate damage to the anterior capsule, as determined by preliminary single exposures.

Thirteen rabbits (of which 3 were albinos) and 3 guinea-pigs have been exposed at distances of 4 inches to 3 feet over periods varying from 2½ to 12 months. All were young animals at the commencement, and in each case the left eye was exposed, the right being used as control.

They may be grouped into three classes according to the degree of exposure and the severity of the inflammatory reaction which follows, the depth of corneal opacity being the most useful criterion of the latter.

Results.

Class I: No Corneal Opacity.—In this group 5 rabbits and 3 guinea-pigs were exposed for from 1 to 3 hours weekly at 3 feet for 3 to 9 months. They were killed 1, 2, 3, and 4 days after the last exposure. In each case there was slight chronic conjunctivitis, the media were clear, and the anterior lens capsule was normal on microscopical examination.

Class II: Slight Corneal Opacity.—Four rabbits were exposed for 1 hour every 10 days at 4 inches for 3, 7, 10, and 12 months respectively. They were killed 4, 7, 7, and 13 days after the last exposure. In each case there was marked chronic conjunctivitis with thickening of the lids and slight ectropion. There was moderate corneal and no lenticular opacity. In Rabbit No. 3, exposed for 12 months, there was a ring-shaped zone of cell proliferation in the pupillary area of the anterior lens capsule (see Plates 6 and 7). The latter was normal in the other three animals.

Class III: Dense Corneal Opacity.—Four rabbits were exposed for 3 hours every 2 weeks at 4 inches for 3, 9, 10, and 11 months respectively. In each case there was chronic conjunctivitis with marked ectropion. The cornea showed a dense opacity which had undergone vascularisation. The lens was clear, and the anterior capsule normal. The animals were killed 1, 2, 7, and 10 days after the last exposure.

With one exception, therefore, this series shows no change in the lens or its capsule as the result of long-continued exposure to rays of short wavelength. With regard to this exception two possible explanations may be advanced.

1. It is an example of a condition described by Hess (Ref. 10). In 1907

Exposure.	Rabbit.	Duration.	Fre-quency.	Distance.	Length of exposure.	Time after exposure at which fixed.	Lids.	Conjunctiva.	Cornea.	Lens (ophthalmoscopically).	Capsule.
		months.	days.	inches.	hours.						
Heavy ...	1	11	14	4	3	24 hours	Marked ectropion	Marked chronic conjunctivitis	Dense opacity, vascularised	N.	N.
„ ...	14	8½	12	4	2	2 days	„	„	„	N.	N.
„ ...	10	10	14	12	3	7 „	„	„	„	N.	N.
„ ...	22	3	12	3	3	7 „	„	„	„	N.	N.
Medium	9	10	10	4	1	4 „	Slight ectropion	„	Slight opacity	N.	N.
„	12	7	10	4	1	13 „	„	„	„	N.	N.
„	3*a*	12	21	4	2	7 „	Marked ectropion	„	„	N.	Marked proliferation.
„	24	3	10	3	2	1 day	„	„	„	N.	N.
Light......	5	3	6	36	3	3 days	N.	Slight chronic conjunctivitis	Clear	N.	N.
„	4	4	6	36	2	24 hours	N.	„	„	N.	N.
„	6*a*	2½	3	36	1	2 days	N.	„	„	N.	N.
„	8*a*	9	6	12	1	4 „	N.	„	„	N.	N.
„	11	7½	10	12	2	13 „	N.	„	„	N.	N.
	Guinea-pig.										
„	1	8	7	3	1	4 „	N.	„	„	N.	N.
„	2	8	7	3	1	4 „	N.	„	„	N.	N.
„	3	7½	6	3	1	4 „	N.	„	„	N.	N.

N, normal. *a*, albino.

he showed that following heavy exposures to the Uviol-glass mercury-vapour lamp, which emits rays down to a wave-length of 253 $\mu\mu$, degeneration of the capsule cells in the pupillary area occurs, followed two to four days later by regeneration as evidenced by the occurrence of mitoses. Surrounding the damaged area, *i.e.* at the pupillary margin, a zone of darkly stained nuclei is seen, an appearance which he describes as an epithelial "wall." That this phenomenon cannot adequately explain the condition of the capsular thickening described in Rabbit No. 3 is shown by three points:—

(*a*) The epithelial "wall" described by Hess is a narrow ring surrounding a wide circle of damaged cells. The zone of proliferation described in Rabbit No. 3 is a wide one enclosing a small area of normal or dividing cells.

(*b*) The epithelial "wall" appears to be due to a swelling of the cells and a tendency of both nuclei and cell protoplasm to stain more deeply than usual. In Rabbit No. 3 the increased density of staining is present to a slight degree but the proliferation has resulted in a layer two or three cells thick.

(*c*) In no other case has cell destruction and regeneration followed an exposure of such slight severity.

2. It is the first stage of the formation of an anterior capsular cataract. While this was the only animal of the series in which capsular proliferation occurred, it is possible that its absence in the other animals may be ascribed to their falling into one of two classes:—(*a*) That in which the short exposure was a subminimal stimulus to cell division; (*b*) That in which the corneal opacity following heavy exposure acted as a shield to the subjacent lens epithelium.

It is suggested, therefore, that in this case the light has acted as a stimulus to proliferation of the normal anterior capsule cells, without them going through the preliminary stage of damage, such as occurs after exposures of greater intensity. With this exception, the results of repeated exposure to the Kromayer lamp agree with those of Birch-Hirschfeld (Ref. 8), who exposed dogs at 10 cm. from Uviol mercury-vapour light for 10 minutes weekly for 18 months, and found no lenticular opacity or capsule changes. As they developed no corneal opacity they fall into line with the first group of my series.

III. PRELIMINARY REPORT ON THE TRANSMISSION OF HÆMOLYSINS TO THE AQUEOUS AFTER EXPOSURE TO SHORT WAVE-LENGTH LIGHT.

Following an exposure to the Kromayer lamp of an intensity such as, in the second series, was repeated frequently over periods of several months, no signs of irritation of the iris or ciliary body are observed by the ordinary methods of physical examination, with the exception of a transient contraction of the pupil and hyperæmia of the iris. At the end of the series of exposures, both iris and ciliary body were normal on microscopical examination, so that to determine if a slight ciliary irritation persisted in the intervals between successive exposures a more delicate test of such irritation had to be employed.

For this purpose advantage was taken of an observation of Römer's (Ref. 15) that the aqueous of animals which had been sensitised to the erythrocytes of a foreign species had no power to hæmolyse the blood of that species. If, however, when the anterior chamber had reformed, a second paracentesis was done, the aqueous obtained was actively hæmolytic. Similarly, if slight iridocyclitis was induced the same result followed.

Rabbits were rendered hæmolytic by injection with washed cats' corpuscles intraperitoneally in doses of 5, 10, 15, and 20 c.c. at intervals of four days. Hæmolysis is most active 8–10 days after the last injection.

Römer's observations were first confirmed as follows:—

First aqueous, 9 cases .... No hæmolysis.

Hæmolysis following simple paracentesis:—

Present 1, 4, 6, 7, 8 days later.

Hæmolysis following slight injury to iris by point of paracentesis needle:—

8 weeks later .... Slight hæmolysis.
10 " .... No "

Hæmolysis following Exposure to Quartz Mercury-vapour lamp.
Left eye exposed. Right eye control.

Exposure.	Paracentesis.	Hæmolysis.	
		L.	R.
$\frac{1}{4}$ hour at 4 inches	6 hours later	Nil	Nil.
$\frac{1}{2}$ " "	$5\frac{1}{2}$ "	"	"
1 " "	5 "	Faint, positive	"
6 hours " "	At end of exposure	Positive	"
1 hour " "	1 day later	"	"
2 hours " "	3 days later	"	"
3 " "	3 "	"	"
3 " "	4 "	"	"
3 " "	6 "	"	"
3 " "	8 "	"	"

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III. *Hæmolysins.*

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DESCRIPTION OF PLATES.

PLATE 4.—Absorption Spectra of Media of Eye (Visible Region).

A. Carbon arc.
B. Carbon crater (base).
C. Cornea.
D. Lens (suspended in saline).
E. Lens (thin layer in parallel-sided cell).
F. Cell containing saline.
G. Vitreous (3/16 inch thick).

Plate 5.—Absorption Spectra of Media of Eye (Ultra-violet Region).

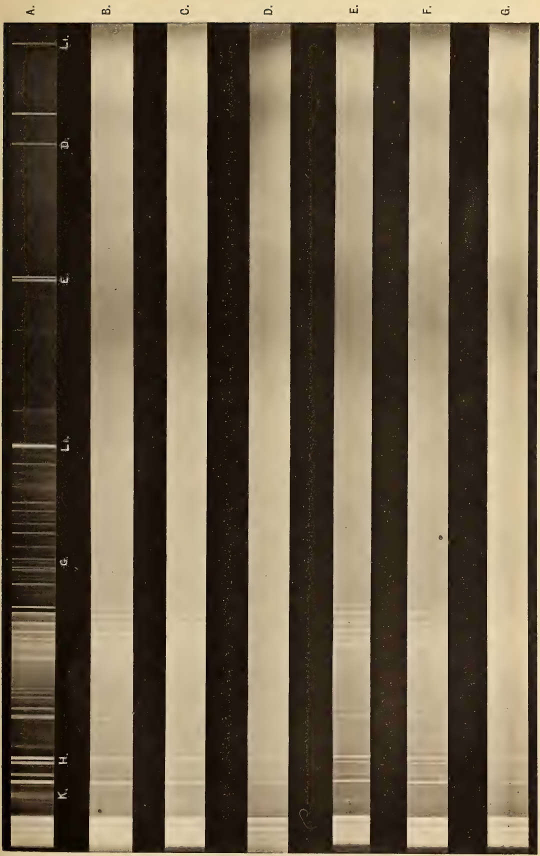
A. Iron arc ; 2 secs.
B. Cornea ; 5 secs.
C. Lens ; 3 secs. ; thin film in quartz cell.
D. Lens ; 1 sec. ; film 1/16 inch thick.
E. Lens ; 5 secs. ; film 2/16 inch thick.
F. Lens ; 80 secs. ; suspended in saline.
G. Vitreous ; 30 secs. ; 3/16 inch thick.
H. Vitreous ; 3 secs. ; 3/16 inch thick.

Plate 6.

Rabbit No. 3. Anterior lens capsule of normal unexposed eye. The number of mitoses present is indicated in the key above by dots ; the position of the fields drawn is indicated by circles. (Zeiss D, Oc. 4.)

Plate 7.

Rabbit No. 3. Anterior lens capsule of exposed eye. The dots in the key represent the distribution, not the number, of mitoses. The left field shows normal cells under cover of the iris ; the central field shows dividing nuclei at the pupillary margin ; the right field shows the greater cell-proliferation where the stimulus is of maximal intensity. (Zeiss D, Oc. 4.)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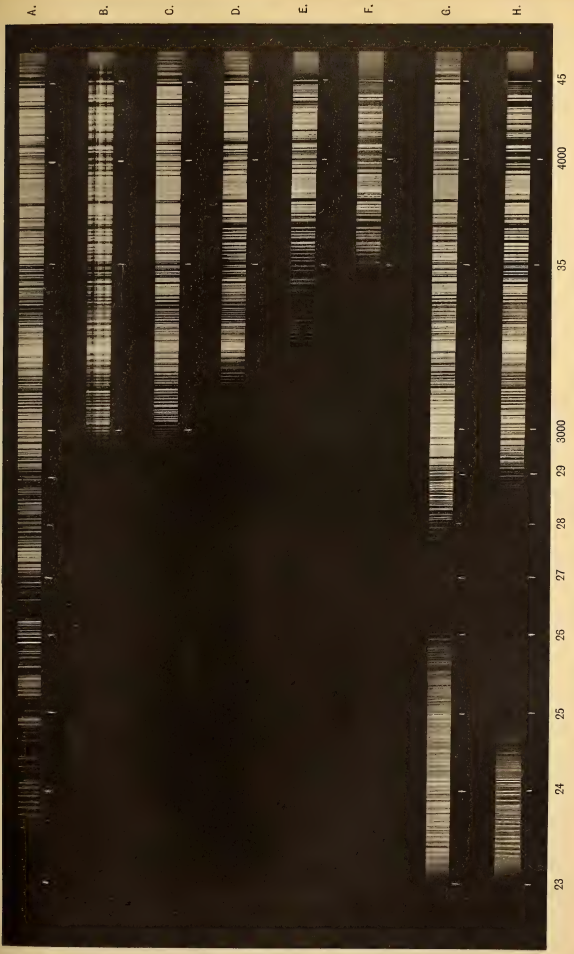
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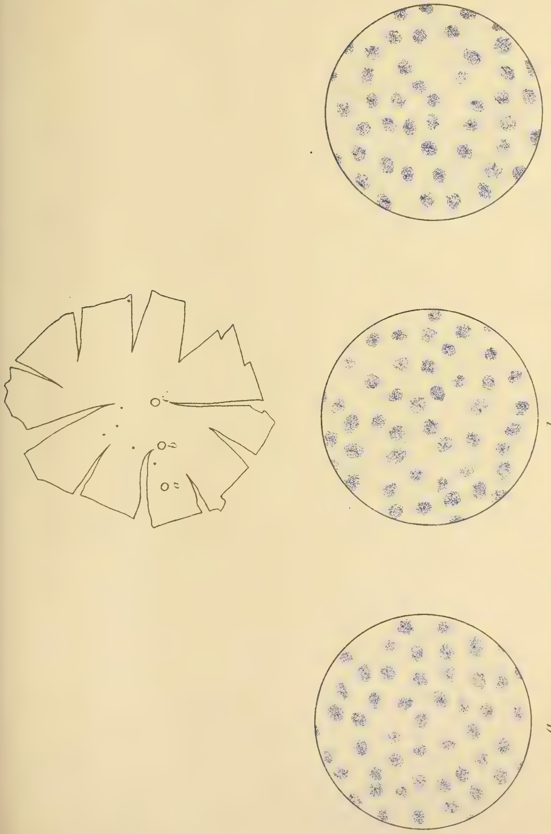
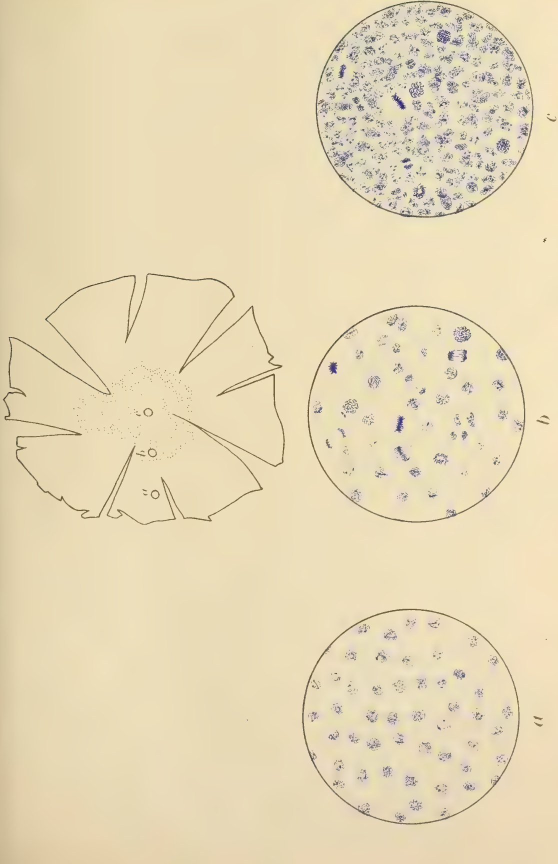


Rabbit Nº 3-R. Normal.



Rabbit Nº 3-L. Exposed.

The Electrical Conductivity of Bacteria, and the Rate of Sterilisation of Bacteria by Electric Currents.

By W. M. Thornton, D.Sc., D.Eng., Professor of Electrical Engineering in Armstrong College, Newcastle-on-Tyne.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received January 12,— Read March 14, 1912.)

1. *Introduction.*

Electrical currents, both alternating and direct, retard the growth of bacteria in liquids through which they are passed, and under certain conditions cause complete sterilisation. The cell-contents are coagulated by the heat generated, or by electrolytic effects within or without the cell. There is the further possibility that protoplasm may be disintegrated by the mechanical action of an alternating current upon molecular charges, similar in effect to that of rapid vibration, which is known to check the growth of, and even to kill, bacteria. Whether the retardation of growth is regarded as the result of changes in the cell or liquid, the effect is largely controlled by the relation between their electrical conductivities. When these are the same the current flows as if the cell were not present, otherwise the current-density in the bacteria is greater or less than that in the liquid, according as their conductivity is respectively the greater or less. In order, therefore, to control effectively the bactericidal action of electrical currents in a liquid, the relative conductivities of the liquid and the contained bacteria should be known. A full summary of the industrial applications of electrical currents in organic processes is contained in Lafar's 'Technisches Handbuch der Mykologie.'* In most of the cases referred to the currents were weak and the voltage gradients in the liquid low. The special feature of the present work is that the voltage and current were taken to their highest limit, under the condition that the temperature did not exceed 30° C., with the testing cell cooled by immersion in running water.

The 'Comptes Rendus' of April, 1896, contained a short account of observations by S. Lortet on the orientation of bacteria in water through which an alternating current was passed. It was stated that only living bacteria orientated, an observation which, if it had been confirmed, would have had bearing on the relation between electricity and life. This is, however, not the case, for bacteria which have been boiled for several hours orientate more freely than before. Lortet's observations would be at once explained if his emulsion had been sterilised by the addition of an ionising liquid, such as perchloride of mercury, the conductivity of which is greater than that of the germs. The fact is that dead or living bacteria orientate equally well.

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* 1899, vol. 1, p. 455.

[*April 24th.*—In this paper the cause of the observed orientation is considered to be the influence of the electric field upon charges induced on the surface of the bacteria. The surface density σ of charge on the interface between two media of resistivities ρ_1, ρ_2, and dielectric constants k_1, k_2, with a current density i normal to the surface, is given by the relation $4\pi\sigma = (k_1\rho_1 - k_2\rho_2)i$. Thus, when k is about 80, as for water, ρ 100 for saline solutions, and i 1 ampère per square centimetre, σ is of order unity.* This is comparable with the surface density on metallic conductors charged to several thousand volts in air, and is much greater than that on a surface between good conductors.

The dielectric constants of saline solutions have still to be experimentally determined; the product $k\rho$ can, however, be found from existing data in terms of the refractive index. The dispersion terms in the Helmholtz–Ketteler formula for the dispersion of light contain the number of electrons N in unit volume as a factor, and since electrical conductivity is also proportional to N, the sum of these terms can be written b/ρ in low frequency fields.

Thus when the formula is applied to solutions of varied conductivity $n^2 = k + b/\rho$, or $k\rho = n^2\rho - b$, n being the refractive index. From tabulated data† the conductivity of saline solutions is nearly proportional to the percentage g of added salt, and we may write $g\rho = a$, a constant. Schütt has shown‡ that for these solutions the change of refractive index is also proportional to the added salt, so that $(n-n_0)/g =$ a constant, c. Thus $\rho = ac/(n-n_0)$ and $k\rho = acn^2/(n-n_0) - b$.

The values of $n^2/(n-n_0)$ for different strengths of solution are as follows:—

g.	$n^2/(n-n_0)$.
1	1010
5	204
10	103
30	36

The product $k\rho$ therefore decreases as salt is added. In the expression for σ the current density is proportional to the strength of the solution, so that, on the whole, if the effect were determined by transfer across one surface only, σ should be higher in strong solutions. But, in the case of a freely suspended body, there will be no orientating couple when the conductivities of the liquid and body are the same, for the current flows through both at the same density everywhere. It is then reasonable to conclude that orientation ceases in a solution having the same conductivity as that of the contained bacteria.

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* See Jeans, 'Electricity and Magnetism,' p. 336.

† See Whetham, 'Theory of Solution,' p. 413.

‡ See Landolt and Börnstein, 'Tabellen,' p. 684.

It has been suggested that, since the observations of orientation had been made on thin layers of liquid, it might equally well be explained by electrical endosmose. This is to some extent met by the fact that orientation is quite as marked in deep hanging drops, and in cells several millimetres deep, filled with distilled water. Observations are difficult in these cases on account of continuous thermal streaming in the liquid. It can, however, be readily shown that endosmose is only of importance at low frequencies, and that at frequencies of several thousand a second it entirely disappears. Orientation was first observed in high frequency fields, and is quite different in character from the rigid oscillatory motion caused by endosmose at low frequencies. At 80 periods a second, vigorous movement of all floating matter was observed. Elongated particles instantly moved into line with the flow, and spherical particles, such as carmine, were carried to and fro with an amplitude of about two of their diameters, thus resembling short rods in the field of vision.

The characteristic feature of the effect was the extreme rapidity with which orientation took place. On substituting an induction coil, with a condenser across the hammer make and break, for the transformer previously used the effect entirely ceased, though the voltage across the drop was now about four times as high. Purified asbestos was then ground to such a degree of fineness that particles were just visible with a No. 4 ocular and 1/12 inch objective. These remained in perfect definition when the current from the induction coil was passed through the liquid. Bacteria which were almost invisible in low-frequency fields owing to the rapid oscillatory movement, remained in perfect definition in the coil current and were at the same time actively orientated.

It may then be said that the mechanical movement of the liquid as a whole under the cover-slip has an amplitude of less than microscopic dimensions at the frequency of the high-tension side of an induction coil, under a gradient of about 1000 volts per centimetre in the film.

As a further test, a strong emulsion of *B. coli communis* being under observation with many hundred slightly motile bacteria in view, the voltage from the coil was applied, and all were seen to be orientated. The primary current was then gradually reduced and the control of the bacteria observed to be weaker, until a point was reached where, though the majority lay in the same direction, at least one-third did not appear to be under control. On raising the voltage all again came into line with the field.]

2. *Method of Measuring the Conductivity of Bacteria.*

From the nature of the case it would be very difficult, if not impossible, to measure the conductivity of bacteria at all accurately by any of the methods at present in use, for example, by passing a current through a fine tube filled with a paste of organisms. Since, however, there is no couple when the conductivities of a rod and the liquid around it are the same, it is only necessary to make trials in a series of liquids of gradually increasing or decreasing conductivity, and to note that in which a rod inclined to the field ceases to show orientation when the current is made or broken.

To make the measurements of resistance in the present case, a series of 12 solutions of sodium chloride in water was made ranging from 0·0016 to 0·04 grm. of salt per cubic centimetre, of which the corresponding conductivities were measured. A drop of the weakest of these was placed upon an ordinary glass microscope slide. To this was added a few bacteria from a pure culture, and a cover-slip dropped over it. The drop was large enough for liquid to exude from the edges of the slip to permit the introduction of platinum wires flat along the edges of the cover-slip in the liquid. The bacteria then being quickly under observation in the microscope, an alternating current was passed and any movement of orientation noted. When they turned into line with the field their conductivity was taken to be higher than that of the liquid. A fresh drop would then be taken from the next higher strength solution, or if the orientation was vigorous from the highest, and the process repeated until that solution was found in which the movement ceased. In the case of high-resistance bacteria a difference of 0·5 mgrm. of salt per cubic centimetre in the solution could be detected in this way.

In most of the experiments a small laboratory induction coil was used. The voltage between the platinum poles when the current was passing was at least 100, giving a gradient in the liquid of 50 volts per centimetre. This was sufficient to show orientation in distilled water. For demonstration it is more satisfactory to use a quarter inch spark induction coil, the voltage of which falls to a few thousand when connected across the drop.

The following table gives the results of the measurements on bacteria which are to be found commonly in water or milk. A few others are given of which there were active growths available at the time. The culture medium is mentioned because of the great difference between cultures on or in different media, in the case of *B. typhosus,* for example.

The chief points established by the values found here are :—

(1) The electrical conductivity of any organism is not independent of the culture medium used.

(2) Bacteria which have their habitat in the animal have higher conductivities than those usually found external to it.

(3) The former have, on the whole, a higher conductivity than blood.

Table I.—Specific Resistances of Bacteria.

	Ohms per cm. cube.	Culture medium.
B. coli communis	140	Agar, rather dry.
„ from fresh sewage	82	Agar, moist.
„ „	60	Broth.
B. typhosus	250	Agar, from broth.
„	200	„ from agar.
„	105	Gelatine, from broth.
„	43	Agar.
„	120	Boiled 1 hour, 200 ohms before boiling.
„	35	Autoclaved 5 hrs., „
B. enteritidis sporogenes	200	Agar.
Sewage streptococcus	45	„
Proteus vulgaris	86	„
B. violaceus	340	„
Micrococcus prodigiosus	340	„
B. subtilis	350	„
B. anthracis	200	„
B. acidi lactici	200	„
„	80	Broth.

In order to see how this variation depended upon the resistances of the media themselves, the following measurements were made:—

Table II.—Specific Resistances of Culture Media, Milk, and Water

	Ohms per cm. cube.	
Nutrient beef agar	60—70	
Beef gelatine, 10 per cent.	115	
Turnip gelatine	275	
4 per cent. gelatine in water	290	
Broth	70—104	No two samples from different stocks the same.
Blood serum	96	Separated by standing.
Fresh blood	200	
Fibrin	245	

Table II—*continued.*

	Ohms. per cm. cube.	
Fresh milk, 1 to 2 hrs. after milking...	233	From different sources.
,, ,, ...	240	
,, ,, ...	252	
,, ,, ...	252	
Fresh cream	557	
Cream with acid reaction	480	
Separated milk	210	
Milk with slight acid reaction.........	196	
Sour milk	180	
Sour and semi-solid	169	
Tap-water, with bacteria added	1400	
Stream water	1300	
Town sewage	1250	

Comparing Tables I and II, it will be seen that *B. typhosus* from gelatine has practically the same conductivity as the culture medium, and that that on agar, though at first high, falls even below that of the medium, suggesting that there is marked absorption of conducting salts by organisms grown on agar.

3. *Influence of Sub-culturing on Conductivity.*

It was recognised from the above figures that, to obtain perfect comparison of the conductivities of various organisms, they should be taken from cultures, not only of the same age, but of the same order of sub-culture. An organism which has been many times sub-cultured could not in general be expected to have quite the same conductivity as one fresh from the animal. The following determinations were made to decide this point. The organism used was *B. coli communis* from house sewage, and the cultures were all in broth from the same stock:—

	Ohms per cm. cube.
Fresh from the animal	95—90
First sub-culture.........................	60
Second ,, ...	45—40
Third ,, ...	40
Fourth ,, ...	35
Fifth ,, ...	35
Sixth ,, ...	35
Seventh ,, ...	35

There is, therefore, a rapid increase in conductivity due to the change of habitat from the body to broth. After the fourth sub-culture a steady state is reached. The ratio of the specific resistance of the medium to that of the organism in broth, taking a mean value of 87 ohms per centimetre cube for the latter, is 87/35, that is 2·5. Taking the value for blood, which is also Waller's value for muscle, as that for the host as a whole, the ratio is 200/90, that is 2·2. The numerical coincidence is probably accidental, but it suggests that there may be, within limits, an adaptation of the organism to its surroundings, such that its electrical conductivity is always the greater.

4. *The Influence of Unidirectional Currents.*

On Water.—In the experiments about to be described all possible care was taken to control the temperature rise consequent upon the passage of the current. With the exception of those on the influence of alternating current on milk, the temperature of the liquid did not rise above 30° C. In the case of water a long and narrow form of sterilising cell was used, to keep the cell cool and at the same time to avoid the influence of any gas liberated at the electrodes when using direct current. Two No. 1 microscope cover-slips, each 2·5 cm. wide and 9 cm. long, were cemented together with paraffin wax to form a V trough 0·15 cm. wide, open at the top. Narrow strips of platinum foil were inserted at each end, the greatest distance between them being 4·5 cm. Even with the highest unidirectional pressures used, *i.e.* 480 volts, across the cell, and a current of 0·5 ampère per square centimetre in it, the fine bubbles of gas which escaped slowly from the electrodes could not be traced more than 0·3 cm. on the surface towards the centre of the cell. The stream of cooling water was on a level with that inside. The resistance between the poles with tap water in the cell was about 50,000 ohms. Quantities of ½ c.c. of an emulsion of *B. coli communis* in non-sterilised tap water were introduced into the trough and exposed to a voltage gradient of 130 volts per centimetre for various intervals of time. With young fresh cultures an exposure of one minute caused slight but perceptible diminution in the number of colonies on a Petri dish culture compared with a control plate.

An exposure to direct current for five hours at a voltage gradient of 210 per centimetre and a current density of 0·3 ampère per square centimetre reduced the colonies in a ratio approaching 100 to 1. After 24 hours' exposure one colony developed, the control plate having a growth too dense to be counted with certainty.

The most efficient form of cell for use with direct current is one in which oxygen liberated at the positive pole bubbles through the liquid in escaping. For this purpose a cell with a short distance between pole plates placed in a horizontal plane in the liquid is convenient, taking a large current at low voltage. The effect is similar to that produced by the passage of ozone through water, and being chemical rather than electrical is not considered here in detail. It provides, however, an effective means of sterilising potable water, using carbon electrodes, which might be of service where electrical power can be had at low rates.

On Milk.—In order next to examine the influence of direct current on milk, and as before to keep the gases at the electrodes from the central portion, a glass cell was made consisting of two circular pole-pits 4 cm. diameter, joined by a tube 1·5 cm. diameter. Flat platinum foil electrodes were used, the distance between them being 10 cm. Filled with fresh milk and connected to a 100-volt supply from secondary cells, a current of 0·15 ampère passed. The current density at the poles was then 0·03, and at the centre of the connecting tube 0·1 ampère per square centimetre. After a few minutes the milk in the neighbourhood of the positive terminal began to curdle, whilst that at the negative pole did not, though the surface became covered with a fine froth. After a quarter of an hour the milk in the positive pole-pit was a complete mass of hard curds, that at the negative a yellowish whey with no sign of curdling. In the centre tube the milk remained fresh and sweet to the taste; the taste of the curdled milk was strongly acid, and of the whey unpleasantly metallic.

These effects were due to direct ionisation, not to the passage of the current, or to the gases liberated, for on passing oxygen or hydrogen from storage cylinders for long periods through milk no curdling or separation resulted, and the milk in the centre tube carried the whole of the current without change, even with 500 volts across the cell. The formation of curds at the positive pole is in agreement with Hardy's conclusion* that coagulation is set up by contact with ions of the opposite sign to that of the liquid. In the present case the negative charge, though this is not so uniformly distributed as in blood cells, can be directly observed under the microscope, using a thin layer of milk and a strong electric field.†

These results show that unidirectional currents can be passed through milk without curdling it except quite near to the electrodes. As a practical process of sterilisation this would, however, be very inefficient on account of the large proportion wasted, and to attempt it at all, voltages of the order of 1000 to 2000 would have to be employed.

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* See Whetham's 'Theory of Solution,' p. 399.

† See 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 638, 2, for the method.

5. *The Sterilisation of Water by Alternating Currents.*

Several observers working with weak current densities have stated that low frequency alternating currents do not affect bacteria,* and there do not appear to be any recorded cases of sterilisation by them other than of d'Arsonval and Charrin,† who found that *B. pyocyaneus* in a flask of liquid placed in the core of a coil carrying an alternating current lost its chromogenic power after 20 minutes' exposure to the induced magnetic field. Whether this was owing to the weak eddy currents set up in the liquid or to the action of the field was not determined. It is, however, improbable, in view of the high current densities required to produce marked effects, that the necessarily feeble eddy currents could have been the cause, which must rather be regarded as a direct action of the magnetic field upon the molecular structure of the cell contents of the particular organisms used. Strong alternating magnetic fields do not appear, from a few trials, to have any characteristic effect on bacteria in general. In making comparative experimental trials of the rate of sterilisation in this way, it is very necessary to use bacteria from the same culture, if possible from the same colony, both on account of the variation of the conductivity discussed in the next section, and of a difference found between bacteria from active and poor growths in their rate of sterilisation by electric currents. *B. coli communis*, many times sub-cultured, was exposed to an alternating current at a frequency of 80 per second and at a constant pressure gradient of 65 volts per centimetre in common salt solution having a resistance of 50 ohms per centimetre cube, in one of 200 ohms resistivity, and in one of 500 ohms. They were each exposed for three hours. The ratios of the number of colonies per unit area on the control plates to that on the exposed plates were respectively 150, 11·0, and 17·5. The rate of sterilisation in this case of constant voltage therefore falls rapidly as the resistance increases. The ratio of the reduction ratio to the current is the same in the first and last of these. The resistance of the bacteria was not measured in every case, but may be taken to be between 35 and 60 ohms per centimetre cube.

If, instead of having the same voltage gradient in each trial, the current-density is kept constant, results are obtained which show very clearly the greater influence of higher voltages. Exposures were made in 50, 200, and 1400 ohms solutions, with a current-density of 0·3 ampère per square centimetre in each case. The reduction ratios were respectively 9, 13, and 26. The bactericidal effect, therefore, does not depend only upon the quantity of electricity passing, but also on the influence of the field, which, though rapidly alternating, may, when high, cause more ionic movement through the cell walls of the organisms, or may act directly by breaking up their protoplasm.

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* Zeit ('Journ. Amer. Med. Assoc.,' Nov., 1910) found that weak alternating currents favoured growth.

† 'Comptes Rendus de la Soc. de Biol.,' 1893, p. 467.

The voltage gradient used in the later experiments approached unity in electrostatic measure. The force on a charge e ($4{\cdot}6 \times 10^{-10}$ E.S.U.) has the same arithmetical value as e in dynes, in unit field. This gives, assuming one such charge upon a section of 3×10^{-8} cm. diameter, an average molecular diameter, a stress of 635 grm. per square centimetre, which is enough, when rapidly reversed, to mechanically disintegrate most soft organic tissues, and presumably protoplasm.

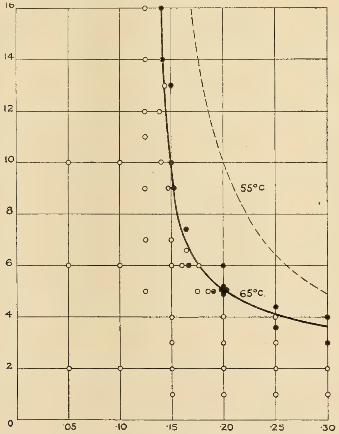
That it is possible to sterilise water by alternating currents at high voltage gradient and current density was shown by exposure for 19 hours at a voltage gradient of 150 per centimetre and a current density of 0·3 ampère per square centimetre. One colony developed as compared with a dense growth on the control plate. A comparison of the effects of direct and alternating current when precautions are taken to avoid the influence of electrolytic products shows that they are very similar. The conditions as to length and section of cell and to constant cooling must be observed.

The results in tap-water were so little different from those in weak salt solutions that the latter may be taken as representative of the degree of sterilisation in tap-water. The effect of time of exposure in the case of *B. acidi lactici* was examined by comparing plates sown with emulsion after 4, 10, and 20 minutes' passage of the current through it, with a control plate. The numbers of colonies are respectively 8000, 300, and 20, the growth in the control entirely covering the agar. This organism is therefore more sensitive to the current than *B. coli communis.* Later results with older cultures do not show so marked a difference, though it is always similar. The chief interest of this lies in the fact that with a current density of 0·5 ampère per square centimetre in cold water, 20 minutes does not cause complete sterilisation, whereas with new milk a shorter exposure does so. One reason for this, apart from heating and cooling, may be that in the latter case the bacteria were just beginning to grow, and were therefore more sensitive to the influence of the current.

6. *The Sterilisation of Milk by Alternating Current.*

For the purpose of exposing milk to alternating currents two large platinum crucibles were arranged, one inside the other, to form a cell, the distance between them at the bottom being 0·7 cm. The interspace could be filled through a hole in the ebonite cap with a known quantity of liquid.

A thermometer was afterwards inserted, and could be readily removed to take samples with a pipette. It was found necessary, on account of the heat produced by the passage of the current, to pack the outer crucible in ice, or, when the stronger currents were used, in a freezing mixture. The temperature was never allowed to rise above 65° C. in one set of trials or 55° C. in another. Working at the lower current densities it was possible to prevent the temperature exceeding the above limits for the whole time of exposure, without stopping the current; but with the higher currents this could not be done, and the time at 0·3 ampère per square centimetre, for example, of three to four minutes, was made up of half-minute runs with the same time for cooling. About 70 exposures were made, of which 55 are given for the 65° C. limit. Cultures were taken as sterile when they showed no growth after 24 hours' incubation at 37° C.



The results given previously of prolonged exposure to alternating currents at current densities of the same order as the above prove that sterilisation obtained by a few minutes' exposure, accompanied by rapid changes of temperature, must be regarded as thermal rather than electrical in origin. It will, however, be seen that the form of the curve follows closely a rectangular hyperbola with a vertical axis displaced to about 0·05 ampère per square centimetre. There is, according to this, a current density at which no sterilisation occurs, however prolonged the exposure, which may explain Zeit's results, the warming action of the current encouraging growth. On the other hand, the fact that the curve is hyperbolic in contour would suggest that the result depends in some measure upon the quantity of electricity passed through the liquid, since this is the same for each point on the curve, if reckoned from the displaced vertical axis. It is probable that the ultimate cause of the results recorded here is not simple, but is in part thermal, and in part direct electrical action upon the molecular structure of the organism. That it is largely thermal is shown by the fact that longer time is required for sterilisation when the temperature is kept at a lower maximum. On the other hand, if it were entirely thermal, the time should be inversely proportional to the square of the current, which is not the case under the conditions of the experiment.

7. *The Action of Ultra-violet Light on Bacteria.*

The results of Sections 4, 5, 6, show that there is not a wide difference between the bactericidal influence of direct and alternating currents when the frequency of the latter is low. When, however, bacteria are exposed to electrical oscillations at their highest possible frequency, namely, that of ultra-violet light, the power required for sterilisation is so small that the result can only be caused by selective absorption of energy, either by the organism as a whole, or by its molecular structure. Exposure of *B. typhosus* in a thin hanging drop of water to strong polarised ultra-violet light causes no marked orientation, which might be expected to occur if the resonance were with the cell as a whole, for the free period of electrical oscillation on a rod is not the same longitudinally and transversely, and the bacillus would be forced, as it is in lower frequency currents, into such a position that the energy absorbed would be greatest. Under favourable conditions, with bacteria fresh from the animal, feeble though clear orientative response to the stopping and starting of polarised ultra-violet light has been obtained, but from the jerky type of movement, distinct from Brownian movement, it would appear to have been derived from stimulus of the flagella rather than to be an orientation by electromechanical forces. The bactericidal maximum found by Marshall Ward was at a wave-length of 4300 tenth metres, corresponding to a frequency of 7×10^{14}, in the low violet. If another maximum should ever be found near a wave-length of 2730, it would clearly point to the action being molecular. The frequency of rotation of an electron doublet in an atom is of the order* 33×10^{14} a second. An electric charge rotating in an orbit in an alternating field of force is accelerated when the frequency of the latter agrees with, or is an odd sub-multiple of, the frequency of rotation. Thus a rotating electric charge in an atom would be eventually split off by resonance when the alternating field had a frequency of 11×10^{14}, corresponding to the third sub-multiple, or $6{\cdot}6 \times 10^{14}$, the fifth; this is the lower limit of the violet part of the spectrum, at which the bactericidal effect ceases. It is now suggested that Ward's lower maximum of 7×10^{14} may be the result of resonance with the fifth sub-multiple. The occurrence of another maximum at the higher frequency—which should be more clearly marked—would definitely decide this and the nature of the whole effect.

8. *Summary and Conclusion.*

The electrical conductivity of bacteria can be measured by observing their orientation when an electric current is passed through a liquid containing them. The values found range from 35 to 350 ohms per centimetre cube, and depend upon the nature and state of the culture medium. The results of sub-culturing are found to be that the conductivity of the bacteria increases at each step, reaching a steady value at about the fourth sub-culture on agar. Water containing added *B. coli communis* can be completely sterilised by direct currents in several hours at 0·3 ampère per square centimetre. Alternating currents sterilise water nearly, if not quite, as well as direct currents having the same current density. In order to obtain well-marked and constant results, it is necessary to use current densities of the order of 0·3 ampère per square centimetre, and to have a form of cell with a thin film of liquid which can be readily cooled. Milk is curdled by direct current at the positive pole and thinned at the negative pole. It can be sterilised, without "skin" forming, by the passage of alternating current through it, this being largely thermal. The cause of the bactericidal action of light is suggested to be syntony between it and the frequency of electronic movement in the protoplasm.

For the rapid sterilisation of liquids in bulk, such as sewage or potable water, the use of an ozone spray is to be preferred to direct electrical action. When, however, it is required to control bacterial growth in liquids over long periods without change of temperature it can be done by the passage of alternating electric current, or of direct current where electrolytic effects are not important. In either case, in order to obtain marked bactericidal effect it is necessary to work at such high current-densities that external cooling is required to control the temperature of the cell.

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* See W. Sutherland, 'Phil. Mag.,' Sept., 1901, Series 6, vol. 2, p. 273, and J. H. Jeans, *ibid.*, Nov., 1910, vol. 1, p. 422.

The preliminary investigation of the conductivity of bacteria and their behaviour in ultra-violet light was made in the Thompson-Yates Laboratories of the University of Liverpool in the summer of 1901. The author is in particular indebted to the late Sir Rubert Boyce for instruction and encouragement in the earlier work, and to Professor Hutchens, of the University of Durham College of Medicine, in the later stages.

An Investigation into the Life-history of Cladothrix dichotoma (*Cohn*).

By David Ellis, Ph.D., D.Sc., F.R.S.E.

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[Plate 8.]

The name *Cladothrix dichotoma* was first applied to this organism by Cohn in 1873. In 1875 he also founded the genus *Streptothrix* to include an organism (*S. Foersteri*) which differed from *Cladothrix* mainly in the possession of a mycelial habit. In 1887 the genus *Actinomyces* was also instituted by the same writer, to include the newly discovered *A. bovis.* Whatever may be the value of the distinction made by Cohn between *Streptothrix* and *Actinomyces,* there is no doubt whatever about the clearness of the line of separation which he set up between these genera and *Cladothrix.* Unfortunately, later writers have used the term *Cladothrix* to indicate not only the only organism belonging to the group, but also species belonging to *Streptothrix.* As examples may be mentioned the organism described by Cienkowski (3) in 1877, which he describes as having a branched mycelial habit. The same mistake was made by Winter (21) in 1884. Influenced, doubtless, by these descriptions, Macé (14) in 1884 denied the separate identity of *Streptothrix* and *Cladothrix.* In his work he describes the characteristics of *Cladothrix,* and gives, under this name, precisely those defined by Cohn as belonging to the genus *Streptothrix.* The confusion by this time had become fixed, and we find the same mistake in later writers. Thus Günther and Rullmann (10), in 1896, describe as *Cladothrix odorifera* what is obviously a *Streptothrix.* Again, Acosta and y Grande Rossi (1) describe as *Cladothrix invulnerabilis* an organism with a branched mycelium and "aërial hyphal threads." The same indubitable characteristics of *Streptothrix* are to be found in Eppinger's (5) *Cladothrix asterioides*, and in Hesse's (2) and Garten's (9) *Cladothrix liquefaciens*, likewise in the organism described as *Cladothrix* by B. Fischer (7), Kedzior (13), Naunym (16), Tchierchke (19), and Flügge (8). Some investigators had avoided this mistake. The first, since Cohn, was Zopf (22), whose masterly treatise, despite small errors in detail, contains the best morphological account of *Cladothrix dichotoma* which has yet been published. We are also indebted to Büsgen (2) and to Hoeflich (12) for several valuable additions to our knowledge of this organism, and particularly because their observations were taken from pure artificial cultures. To Büsgen belongs the credit of being the first to obtain a pure culture of this species, while the fullness of Hoeflich's account of growth in artificial media leaves nothing to be desired. On the other hand, the researches of Sauvageau and Radais (18) have put our knowledge of the genus *Streptothrix* on a firm basis. We know now that the two groups are, phylogenetically, very far apart, and that, with one exception, *Cladothrix dichotoma* stands alone in the group Cladothricaceæ. This exception is *Sphærotilus natans*; the inclusion of this organism and *Cladothrix dichotoma* into a single group (called Sphærotilus) by Migula (15) is a step the wisdom of which is very questionable.

Distribution.—Cladothrix dichotoma was described by Zopf as the "Wasserpilz par excellence," on account of its very wide distribution. He referred more particularly to the neighbourhood of Berlin. In the neighbourhood of Glasgow the organism does not thrive particularly well. In waters with a high organic content the predominant place is held by one or more species of the lower bacteria, or one of the higher fungi. In two places only was a predominant growth of *Cladothrix dichotoma* found in waters containing organic matter in solution. From one of these, a ferruginous stream running close to Possil Marsh, near Glasgow, the whole of the present observations were taken. The water which forms this stream is collected from a neighbouring cemetery that is situated on a slight eminence. This accounts for the comparatively high amount of organic matter in solution in this water.

Analysis of the Water.—This showed that in all respects the water was of the same character as a very dilute sewage. In addition, it contained a small amount of ferrous salt in solution, and of course a large amount of the insoluble red ferric hydroxide which imparts the characteristic colour to streams of a ferruginous nature.

General Characteristics of Cladothrix dichotoma.—The organism consists usually of long colourless threads of 1–3 μ in width. When undisturbed, the length may reach up to 3 mm. Each thread consists of a single row of rods enclosed in a delicate sheath. The thread may be free, or attached to blades of grass or other similar objects. When free, the thread may be floating or it may assume motility. Its general microscopic appearance is shown in fig. 1 (Plate 8). The rod-shaped cells vary in length, but the average is 3–5 times the width. When the thread is attached the cells at the free end are, on the whole, longer than those at the other end. In some threads the cells may be some distance apart (fig. 2). The falsely dichotomous appearance of this species (fig. 3) is seen only when the stream is moving very slowly or is at a standstill. False dichotomy is due to the growth into threads, of cells which have slipped out sideways. In my own cultures its formation was quite exceptional.

The Sheath.—The sheath forms a hollow tube, closed at both ends when the thread is young (fig. 4). In the older condition, if attached, the thread opens at the apex, when the enclosed cells escape into the surrounding water (figs. 2 and 18). It is generally assumed that the sheath is a delicate but firm textured envelope in the form of a hollow cylinder, and that this condition is maintained throughout the life of the plant. All my observations show, however, that the sheath is a clinging mucilaginous envelope during the early stages of growth and only later assumes a firm consistency. It is developed by a mucilaginous secretion of the walls of the young cells. In very young threads, composed of two or three cells only, there is no trace of a sheath. This structure can best be studied by means of iodine preparations, for the cells become deeply stained, while the sheath, though remaining uncoloured, becomes more opaque; further, the cells contract somewhat, thus making the sheath a more conspicuous object (fig. 5). In normal healthy threads, before the splitting of the sheath at the apex, *there are transverse bars of the same material as the sheath between each cell.* These can best be demonstrated by leaving freshly collected threads in ether for two or three days in order to cause the cells to contract (fig. 6). The contents of the cells will have then become disorganised and the cells themselves noticeably smaller. The same result can be produced by iodine if material at the right stage be selected (fig. 7). These bars are also often seen in old threads that have become partially disorganised (figs. 8 and 9); in such cases the cells decompose faster than the sheath. Previous writers make no mention of these transverse bars and the only indication that I can find of their existence in the works of previous writers is in fig. 17 of Büsgen's (2) publication. On p. 151 he says, "Von anderen Entwickelungsständen der Cladothrix traten in meinen Culturen nur noch eigentümlich gestaltete Fäden

auf, welche sich durch besonders hormogonienbildenden Algen vergleichen lassen. Dieselben bezeichneten sich durch besonders dicke Scheiden und eine auffallende Gruppirung ihrer Stäbchen aus. Oft ebenso lang wie breit, lagen die letzteren beispielsweise in wenigglicderigen Reihen zusammen, welche durch Pfropfen einer structurlosen Masse, wohl Reste abgestorbener Stäbchen, getrennt waren."

If this figure be compared with fig. 10 of this publication, in which two of these broad transverse bands are shown, it will be seen that the mapping out of the "hormogonia" was probably due in his cultures, and certainly in mine, to the formation of very broad transverse bands of the same material as the sheath. But, under appropriate treatment, these transverse bands can be found between each cell. In fig. 10 we see two of these broad bands, viz., at *a* and at *b*. They are obviously formed by the accumulation of sheath material in those spaces left empty by the slipping out of one of the cells through the sides of the sheath. The space occupied by this cell then becomes filled with the same material as that composing the sheath, and so the appearance presented in fig. 10 at *a* and at *b* is gradually formed. When the length of such a band is equal to that of one of the cells, it often has a hollow consistency, the hollow space being obviously that previously occupied by a cell. In old sheaths there is no trace of the transverse bars. This may be explained as follows: it is obvious that the sheath, at first soft, later hardens. The growth of the contained cells however continues, with the result that the apex of the sheath is burst through and the sheath, now hard, remains permanently open. Whilst the sheath is hardening, however, the pressure of the growing cells causes the latter to break through the transverse bars, and as they closely fit the sheath the movements of the growing and dividing cells would soon smooth down all projections caused by the remains of the bars; this would take place whilst the sheath was hardening and, of course, after hardening no more transverse walls would be formed. It is interesting to note that a similar secretion of mucilage, followed by a similar hardening of this material, resulting in its complete separation from the cells has been described by Hansen for yeast-cells (see Klöckers, 'Gärungsorganismen,' p. 174). In such cases the yeast-cells are enclosed inside the meshes of a hardened reticulate mucilaginous network. This hard network is obviously the same kind of material as that forming the sheath of the *Cladothrix* cells.

The Vegetative Cells.—The cells are usually 3–5 times as long as they are broad, though they are shorter in growing cells (figs. 1, 2, 4, 10). The average width is about $1\frac{1}{2}\,\mu$. By the use of stains, the cell is seen to be bounded by a sharply defined double contoured membrane. The best stain for the purpose is iodine. The membrane is coloured a deep brownish red, the cytoplasm a light transparent brown, whilst the sheath remains uncoloured. Methylene-blue, or carbol-fuchsin, or Bismarck-brown may also be used for staining, but iodine is undoubtedly the best. In young cells the cytoplasm is unvacuolated, and in it are usually found some strongly refractive round bodies. These are oil-bodies and have already been noticed by Büsgen (2). In artificial cultures the oil-drops are wanting and in their place we find large empty spaces in the cytoplasm. Hoeflich (12), who worked with artificial cultures, does not mention them, but states that the cytoplasm is strongly vacuolated, hence we may conclude that in artificial cultures the conditions are not favourable enough to enable the organism to store food material. For their observation I have taken samples direct from nature, and examined as soon as possible after collection. This is necessary because the threads decompose very rapidly. A cell may have two or three large oil globules or a larger number of small ones (fig. 11, *b*). In many cells I also found glycogen as a reserve material, either with or without oil-globules (figs. 12, 13). This reserve material was sometimes found as a covering to the oil.

I am strongly of the opinion that the *membrane is often used as a depository for reserve food material.* This is shown by the fact that, when there was much glycogen in the cell, the colour of the membrane when stained with iodine could not be distinguished from that of the glycogen deposited in the cytoplasm. On the other hand, the membranes of cells belonging to threads of a five to six days' old culture, in which glycogen was never found, were markedly free of colour when treated with iodine. It must be borne in mind that the membranes of bacteria and their allies have not the same firm consistency as have those of the higher plants. They are more readily permeable, and solid substances enclosed in the cell can sometimes be seen being thrown out without any apparent difficulty when, during staining, the absorption of certain liquids sets up a high osmotic pressure. In the case of *Spirillum volutans* I have on several occasions observed the ejection of volutin granules during the process of staining. Such membranes are probably well adapted to serve as depositories of food material, and it is a membrane of this kind that *Cladothrix* possesses.

Some interesting facts are brought to light by the observation of threads that are gradually undergoing decomposition. Large gaps are seen in the cytoplasm, marking the spots originally occupied by the reserve food material; the membrane, being more resistant, stands out clearly in such cells, thus giving us perhaps the best evidence of the morphological unity of this organ (fig. 15). In still older threads the individuality of the cells is lost, and finally the whole breaks down, when irregular lumps of cytoplasm may subsequently be seen in the surrounding fluid. These lumps must serve as a valuable source of food to the other micro-organisms in the water.

Hoeflich (12) in p. 61 speaks of vacuoles as though they were obvious constituents of the cell; as he does not mention the presence of oil and worked with artificial cultures, I think it probable that his vacuoles were either oil drops or empty cavities in the cytoplasm. On several occasions I have observed apparent vacuoles in the stained cells of artificial cultures, but as these might just as well be referred to the spaces originally occupied by the food material I have refrained from drawing any conclusions from the fact.

False Dichotomy.—The characteristic tree-like appearance of *Cladothrix* (fig. 3) is assumed only when the water is practically stationary. A cell slips out sideways through the mucilaginous sheath and grows into a thread, the mucilage holds the branch and parent together, and so it appears as if true branching had taken place. In fig. 16 is shown a "branch" attached to the parent thread. At the point of attachment there were no indications of the existence of two separate sheaths. As the cells which give rise to the daughter-thread slip out through a very soft sheath, such is not to be expected, and when later the mucilaginous sheath hardens, at the point of contact of the daughter- with the parent-thread, the sheath would harden as one piece. When the conditions are not restful, the falsely dichotomous growth is not possible, for the mucilaginous sheath has not sufficient adhesive power to prevent the escape of the liberated cell, but, if the conditions are restful, adhesion is possible, and, when the sheath later hardens, the escape of the daughter-threads is rendered quite impossible, even if the water becomes subsequently somewhat less restful. It is, therefore, only under exceptional conditions that the *Cladothrix* of the text-books is formed.

Cell-division.—The stages of cell-division can be followed by reference to fig. 15. There is first a slight elongation of the cell, then a slight constriction in or near the middle, after which a thin transverse membrane is thrown across the cell at the constricted part. The constriction gradually deepens and the membrane becomes thicker; this is followed by a drawing apart of the daughter-cells, after which each elongates until the adult form is reached. The process is thus essentially the same as in the Bacteriaceæ Stained preparations sometimes show that the separated cells are connected by a mucilaginous thread (fig. 17). No doubt, for a time, a thin thread of protoplasm, running through the mucilage, also joins the cells.

When the sheath hardens, the pressure and growth of the dividing cells causes a rupture of the sheath at the apex, after which the sheath remains permanently open. This pressure also causes the side-slipping of individual cells before the sheath hardens, resulting, if the water be moving very slowly, in the cell growing into a "false branch."

Cell-division takes place at first uniformly throughout the thread, but, after the apex has been opened, it is mostly confined to the basal part, as at the apex only full-developed mature cells can be observed.

Multiplication.—This is essentially of one kind, viz., by the rejuvenescence of a single cell or a group of cells. The following varieties of this mode of multiplication may be distinguished:—

1. *Multiplication by Liberation of Thread-fragments.*—This is the mode followed by motile threads, in which I have seen no trace of a ruptured sheath nor of the liberation of single cells. Some of these motile threads may reach to a length of 1–1½ μ, and even in these the sheath is soft, and envelops the cells very closely. The liberation of thread-fragments has been observed by Zopf in the case of attached threads, and I am able to confirm his statements. In my observations, however, the liberated fragments assumed a spiral form. I propose dealing more fully with this in a later section.

2. *Multiplication by the Liberation of Rejuvenated Single Cells.*—On the nature of this mode of multiplication all observers are agreed. Sooner or later, in attached threads, the apex of the sheath bursts, and single cells are pushed out into the surrounding water. These cells are sometimes devoid of motility and drift placidly away, at other times they develop organs of motion, by the aid of which they swim out of the sheath. I have observed the actual liberation of a non-motile cell. It seemed to be thrust out of the sheath by the aid of a small push from behind (fig. 18). There is nothing of a sporogenous character about these cells, for they behave in precisely the same way, as regards growth, division, etc., whether within or without the sheath. They are vegetative cells of the same nature as those of the genus *Bacillus*. Hoeflich follows this line of thought so far as the non-motile cells are concerned, but he refers to the same when motile as "spores," because, previous to ejection, in addition to the development of cilia, certain changes in vacuolisation occur. The changes which he describes seem to me to be connected with changes incident to the development of cilia. The only difference, therefore, between what is confessedly a vegetative cell and what he calls a "spore" is that the latter is motile. These "spores" appear to me to be merely swarming vegetative cells. Hoeflich does, indeed, refer to the germination of these bodies, but, considering the importance of the point, a bare mention of the fact, and a very inconclusive drawing, are not sufficient evidence to warrant full acceptance without confirmation.

The Formation of Spiral Threads.—Since the announcement of Zopf in 1882 of the fact that under certain circumstances *Cladothrix dichotoma* liberates spiral threads which behave like *Spirilla*, criticism on this point has been hostile. Winogradsky (20) was strongly of opinion that the life-cycle of *Cladothrix dichotoma* was completed by the development of "swarming red cells" which reproduced the threads. He professed himself very sceptical as to the formation of spiral reproductive cells, though pointing out that wavy threads were not uncommon in this species, which every investigator of this species has of course observed.

Following Winogradsky, the opinion of all subsequent researchers on this subject has set in the same direction. Büsgen (2) accounts for Zopf's *Spirillum* threads by supposing that a *Spirillum* species was present as an impurity, and this in spite of the fact that Zopf had mentioned (p. 10 of his 'Zur Morphologie der Spaltpflanzen') that he had actually observed these spiral fragments being detached from the thread. Hoeflich (12) takes up the same attitude and denies the existence of spiral threads. The consequence has been that modern writers of text-books on this subject have omitted all mention of these *Spirilla*. Being convinced of the truth of Zopf's observations on this point I have examined hundreds of samples of *Cladothrix* cultures extending over a period of 18 months, in order to be able to confirm his observations.

How the conditions arose I cannot say, but in one culture, and one only I observed the whole procedure as outlined by Zopf. This was in a seven-day-old culture in an open beaker. The medium consisted of a solution of ferrous carbonate to which a drop of 0·05-per-cent. peptone had been added. Between the 7th and the 10th day, the organism fell to pieces. The culture was impure, it is true, but *Cladothrix* threads are unmistakeable after a very little experience. On the 7th day I found the tufts of threads in a state of violent trembling. The surrounding water contained spiral threads that in the unstained condition could not be distinguished from typical *Spirilla*. Cilia preparations showed these to possess polar ciliation. Each was seen to be a *Cladothrix* thread of three to five cells, and with polar cilia. The trembling of the tuft of threads was obviously due to the violent wriggling of these spiral forms in their efforts to free themselves from the colony. Examples of these are given in figs. 20 to 24. Each *Spirillum* had 1 to 3 polar cilia. The *Spirillum* consisted of from half to three wave-lengths. Some consisted of one cell only. *The cilia preparations showed spiral fragments in all stages of liberation.* In fig. 25 is shown one that has been newly liberated. It was not possible to ascertain whether these *Spirilla* permanently retained the spiral form or whether they settled on some object and developed into typical *Cladothrix* threads: probably both sequences followed. Winogradsky, writing on this point, says: "Nur dann konnte man den genetischen Zusammenhang eines Spirillum mit Cladothrix für bewiesen halten, wenn er einen Cladothrix-faden aus einem echten noch gewissermassen unter Beibehaltung seiner characteristischen Eigenschaften sich teilenden Spirillum erzogen hätte." It is proved at any rate that spiral forms with spiral movements and polar cilia are liberated from what was undoubtedly a *Cladothrix* colony. These appear in the surrounding water as organisms in no respect different from what are commonly regarded as *Spirilla.* Their subsequent fate is immaterial to the point at issue, so long as it is proved that they originated from a *Cladothrix* thread. One cannot doubt that sooner or later they settle down and develop into the typical threads. This point has not been proved. If this proof were furnished it would be an interesting addition to our knowledge of this organism, but would have only a slight bearing on the point under discussion: the essential point lies in the proof of the existence of *Spirilla* originating from a *Cladothrix* thread, not in proving what becomes of them after they have been formed.

The Formation of Cocci.—According to Zopf, *Cladothrix* also multiplies by a splitting up of its cells into cocci. I have not observed this method of multiplication. It is common in the case of *Crenothrix polyspora* when growing under unusually favourable circumstances (see Garrett, 'Public Health,' No. 1, 1896), and one must bear in mind that when during active growth the elongation of bacterial cells does not keep pace with their division, the length of the cells in such a culture is not greater than their breadth, and consequently cocci-like rods are formed. It is possible that Zopf may have observed such a phenomenon. As young *Crenothrix* threads resemble *Cladothrix* threads, it is also possible that he may have mistaken the former for the latter. The matter must be regarded as not yet settled.

Synopsis of Methods of Reproduction.—We may, therefore, enumerate the following kinds of reproductive organs as occurring in *Cladothrix dichotoma*:—

a. Rod-shaped swarm *cells.*
b. Spirally shaped swarm *cells.*
c. Rod-shaped swarm *fragments.*
d. Spirally shaped swarm *fragments.*
e. Rod-shaped non-motile *fragments.*

Artificial Cultures.—Büsgen was the first to obtain artificial cultures. He was followed by Hoeflich (12), who gave a very complete and accurate account of the growths of this organism in various media. My own observations on this point were chiefly of a confirmatory nature. In all media growth commences by the formation in the liquid of characteristic grey, easily visible flecks. Microscopically examined, each fleck is seen as represented in fig. 19. This is, I think, the first time that a figure of a *Cladothrix* colony has been published. The presence of a large number of flecks gives the liquid a very turbid appearance. They usually congregate at the top, but fall to the bottom when gently shaken, and remain there. After a few days a flocculent grey deposit is formed. The organism is strongly aërobic, and gradually liquefies gelatine when grown in gelatine nutrient media.

Motility and Organs of Movement.—Hitherto, investigations of the organs of movement of *Cladothrix* have been confined to the cilia of single independent cells. The disposition of cilia in motile threads consisting of more than one cell has not been touched upon. Motile threads of *Cladothrix* are, however, common, and if motile single cells develop into motile threads consisting of many cells, it is obvious that a development of fresh cilia must also take place in order to secure an adequate propelling force. Cilia preparations were first made of single motile cells. The cilia of such cells have been investigated by Hoeflich (12) and by A. Fischer (6). They were shown to be situated in a sub-polar position. Unfortunately, I could not obtain cells in a swarming condition inside the sheath, but I was able to obtain cilia preparations of several immediately after their liberation from the sheath.

In all such preparations the cilia were disposed in the polar and not in a sub-polar position (figs. 26, 27). With regard to the cilia of motile threads, in these also the cilia were placed at the poles of the individual cells. The cilia of a thread of seven cells are shown in fig. 28, of five cells in fig. 20, and of two cells in fig. 27. As the threads become longer the movement gets slower; threads of ½ mm. in length move with a very slow stately movement. Beyond this length movement ceases, evidently because the development of cilia has not been able to keep pace with the increase of mass caused by the growth and development of the cells.

The motility and organs of movement of the liberated motile spiral threads have already been described.

The Organ of Attachment of Attached Threads.—No indication was seen of a special organ of attachment. Büsgen mentions the existence of a small adhesive disc, but in my cultures when attached threads were liberated there was nothing to show which end had been previously attached.

The Phylogenetic Position of Cladothrix dichotoma.—The organism that is undoubtedly most closely allied to *Cladothrix* is *Sphærotilus natans*. The latter differs from the former only in having a very delicate and slimy sheath, and in that a common sheath acts as a covering for a large number of threads. Both Fischer and Migula have united *Cladothrix* and *Sphærotilus* into a single genus, to which the name *Sphærotilus* has been given. As the mucilage secretion must be largely dependent on external circumstances, it is doubtful, I think, whether it could be demonstrated that the points of difference between the two organisms were other than mere local adaptations. In the only figure of *Sphærotilus natans* that I have seen* there was nothing to indicate that the organism was other than *Cladothrix dichotoma.* The organism described in these pages differs more from the normal *Cladothrix* in essential points than does *Sphærotilus natans.* The conclusion seems justified that we are dealing with what Migula appropriately terms a "Sammel-species." *Cladothrix* is like *Bacillus coli communis,* a cluster of sub-species clustered round a central widely distributed form. Migula emphasises this point from a physiological standpoint. The existence of the organism dealt with in these pages and of *Sphærotilus natans* demonstrates the fact that from a morphological standpoint also *Cladothrix dichotoma* must be regarded as a cluster of varieties around a central dominant form.

Amongst other organisms *Crenothrix polyspora* and *Clonothrix fusca* must be regarded as being most closely allied. Their differences are not phylogenetically of great importance. Slightly further removed are *Leptothrix, Gallionella,* and *Spirophyllum*: they differ chiefly in forming reproductive cells by abstriction. At the present state of our knowledge it is unwise to do more than hint at the possible relationships of *Cladothrix* with the other members of the higher bacteria. The sulphur bacteria from a phylogenetic standpoint form a highly unnatural group, and *Cladothrix* is much more closely related to forms like *Beggiatoa* than this is to the other sulphur bacteria, *e.g.*, *Thiophysa* or *Chromatium.*

The relation of *Cladothrix* to *Streptothrix,* using this term as defined by Cohn and as followed by Rullmann in Lafar's 'Handbuch der technischen Mycologie,' is a very distant one. The whole habit of the latter plant, with its mycelial structure and mycelial mode of branching, shows its relationship with the higher fungi, whilst *Cladothrix,* on the other hand, is more nearly related to the Algæ, and, had it possessed colouring matter, there would have been no difficulty in relegating it to that class. Among the Algæ, the Cyanophyceæ is the group to which *Cladothrix* has the strongest leanings. The general resemblance to forms like *Oscillatoria,* to *Hepalosiphon,* and to *Plectonema* is sufficiently striking to arrest attention. On the other hand, the rod-like ciliated swarming cells, the method of cell division, the nature of its reserve material, attest the fact that we cannot place *Cladothrix* very far from the lower bacteria, particularly the genera *Bacillus* and *Pseudomonas.*

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* 'Report of Royal Commission for Disposal of Sewage.'

The connection with the genus *Spirillum* is also indicated by the occasional thrusting forth of spiral cells or thread-fragments in all essentials identical with those belonging to the genus *Spirillum.*

In fact we see in *Cladothrix* a very good illustration of the fact that very low down in the scale of organisation, organisms tend to approach more closely in their affinities. It is in such widely distributed organisms as this that such facts are best demonstrated.

Summary.

Distribution.—Widely distributed on the Continent, but not so in Great Britain, its place being taken by members of the higher fungi.

Occurrence.—Best growth found in ferruginous waters containing a slight organic contamination.

General Characteristics.—Long, colourless threads, 1–3 mm. long. Threads may be free or attached, motile or non-motile. Tree-like colonies, exhibiting false dichotomy, formed only when the water is almost or entirely motionless.

Sheath.—Cells are enclosed in a sheath, which is soft and mucilaginous in the young condition, but later hardens; it is ultimately split at the apex by the growth of the enclosed cells, after which it remains permanently open. The sheaths of motile threads do not harden, and consequently always envelop the cells as a closely fitting sheath. *Between the individual cells, transverse bars of the same material as the sheath are formed.* When the sheath hardens, the transverse bars are destroyed by the pressure of the growing cells.

The Vegetative Cells.—The cells are arranged in a single row inside the sheath, and are $1–1\frac{1}{2}\,\mu$ in width, and, on the average, $4–6\,\mu$ in length. The cell has a distinct membrane, inside which cytoplasm and reserve matter, in the form of oil globules and glycogen, may be distinguished. Small, clearer spaces in the cytoplasm may also be distinguished, but their nature is regarded as doubtful, as they might either be vacuoles or empty spaces left after the removal of the reserve material.

Cell-division.—Is of the same nature as the same process in the orders Bacteriacæ and Coccaceæ; a transverse wall is thrown across, being followed by constriction at the same point; the constriction extends until the two daughter-cells are separated. Cell-division in the attached threads is more active near the base than at the apex, but, in the case of motile threads, is uniformly distributed. As cell-division takes place only in one direction of of space, and transversely to the long axis of the thread, pressure is generated along the long axis of the thread, with the result that the sheath splits open at the apex.

Multiplication.—Entirely limited to the rejuvenescence of single cells or thread-fragments, which become detached and grow into new plants. No sexual or asexual spores are formed. In the case of motile threads, fragments are cut off, which grow into new threads; the same process takes place in the attached threads, the fragments being usually straight, but occasionally spiral in form. Such fragments contain usually from one to seven cells. Another variation of the same method in the case of attached threads is by the liberation, through the open apex of the sheath, of single, either motile or non-motile, cells. These cells grow into new threads. In the species under investigation, both the independent motile cells and the cells of the motile thread-fragments possessed polar cilia.

Spiral Threads.—Under certain conditions of growth *Cladothrix* liberates single cells or fragments of threads, composed of several cells, which immediately on liberation assume a spiral form, move in an undulating manner, and develop polar cilia.

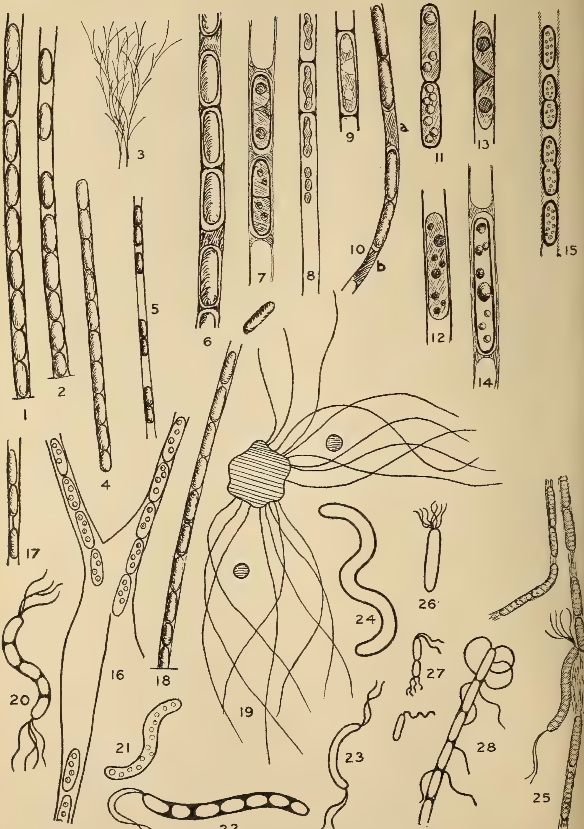
Formation of Cocci.—In the author's cultures such formations were not observed.

Artificial Cultures.—The artificial cultures instituted for confirmatory purposes showed the accuracy of Hoeflich's observations.

Motility and Organs of Movement.—The cells of the variety investigated by the author, when motile, in all cases owed their motility to cilia that were situated in a polar position, thus differing from the Continental varieties, in which the cilia are placed in a sub-polar position. Straight motile threads possessed one to three cilia at the poles of each cell composing the thread. Spiral motile threads, on the other hand, possessed one to three cilia only at the ends of the whole thread, the intermediate cells being devoid of cilia.

Organs of Attachment.—So far as could be observed the attached threads were held only by their own mucilaginous secretions.

Phylogenetic Position of Cladothrix dichotoma.—The most closely allied organism is *Sphærotilus natans*, the connection of which is so close that it must be regarded as a variety of *Cladothrix*. The investigation shows that morphological as well as physiological varieties of this widely distributed organism exist. The relationship to *Streptothrix* is an extremely distant one; in habits, methods of reproduction, and structure, *Cladothrix* is closely related both to the Cyanophyceæ and to the lower bacteria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b

EXPLANATION OF PLATE.

Figs. 1, 2.—*Cladothrix* cells enclosed in hardened mucilaginous sheath. × 2000.

Fig. 3.—Colony showing false dichotomy. × 125.

„ 4.—*Cladothrix* threads enclosed in unhardened mucilage. × 500.

„ 5.—Thread with hardened ruptured mucilage and from which most of the cells have escaped. × 1000.

„ 6.—Portion of thread containing transverse bars of hardened mucilage. × 3000.

„ 7.—A portion of thread stained with iodine, showing transverse bars and glycogen reserve material. × 3000.

„ 8.—Old threads, showing transverse bars of mucilage, and disorganised cells. × 3000.

„ 9.—One cell, enclosed in hardened mucilaginous sheath, showing gaps in cytoplasm, originally occupied by food material. × 2000.

„ 10.—Sheath showing at *a* and *b* broad transverse bars of mucilage. The mucilage has filled up spaces originally occupied by cells. × 1500.

„ 11.—Two cells showing reserve material in form of oil drops. × 3000.

„ 12, 13.—Cells, stained with iodine, showing reserve food in form of glycogen. × 3000.

„ 14.—Portion of thread containing one cell, stained with iodine, and showing reserve food in the form of oil drops and glycogen. The glycogen is superimposed on the oil drops. × 3000.

„ 15.—Old thread, the cytoplasm of which has almost entirely disappeared. Cell walls are less completely disorganised. Well adapted to show stages of cell-division. × 2000.

„ 16.—Somewhat diagrammatic representation of parent-thread to which a daughter-thread is attached. × 3000.

„ 17.—Portion of thread showing two cells, derived by division of a single cell, still connected by a band of mucilage, though a third of their length apart. × 1000.

„ 18.—Thread from which the cells are in process of being liberated through the ruptured apex. × 1000.

„ 19.—Colony of *Cladothrix* threads in artificial nutrient solution. Whole forms a minute gray speck, visible to the naked eye, floating near the surface of the liquid. × 100.

„ 20—22.—Spiral fragments of *Cladothrix*, showing polar ciliation. The spiral thread in fig. 20 is composed of five cells, in fig. 21 of one cell, and in fig. 22 of seven cells. × 1500.

„ 23.—Spirally twisted cell with polar ciliation, liberated laterally from a *Cladothrix* thread. × 1000.

„ 24.—A spiral fragment liberated sideways from a *Cladothrix* thread. × 1500.

„ 25.—Cilia preparation of threads from an artificial culture. Threads were in a state of violent trembling as a result of the liberation of spirally twisted and polar ciliated threads. At *a* is shown a cell on point of liberation, which has elongated slightly, become spirally twisted, and polar-wise ciliated after liberated from thread. × 1000.

„ 26.—Liberated cell, showing a comparatively large number of polar cilia. × 1000.

„ 27.—Newly liberated motile straight rod cells. × 1000.

, 28.—Newly liberated straight thread fragment. The cells of which it is composed possess polar cilia. × 1000.

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Studies on Enzyme Action. XVI.—*The Enzymes of Emulsin.* (I) *Prunase, the Correlate of Prunasin.*

By H. E. Armstrong, F.R.S., E. F. Armstrong and E. Horton.

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[*International Catalogue of Scientific Literature.*
Author's title slip :—D. M. Q.
D 8014 Emulsin (almond). Presence of prunase in
Q 1230, D 8012 } Prunase and the specific nature of enzyme action
D 1850 Prunasin—hydrolysis by prunase.
M 3120 Enzyme of *Prunus laurocerasus.*]

The subject has been dealt with previously in Nos. X, XII and XIII of these studies, in all of which the view was advocated that the resolution of amygdalin into two molecules of glucose and one of the cyanhydrol (CN·CHPh·OH) by almond emulsin is effected through the agency of two distinct enzymes—amygdalase and a *β-glucase ;* the former being the enzyme by which the amygdalin is resolved into glucose and Fischer's glucoside[*] and the β-glucase that by which this glucoside is subsequently resolved into glucose and the cyanhydrol.

"Emulsin" occurs in the almond *fruit* and in the fruits of a variety of allied species of plants *together with amygdalin.* In continuing our studies, we have directed our attention to plant materials containing the simpler cyanophoric glucoside derivable from amygdalin, the primary object we had in view being to institute a comparison between the "enzyme" associated with the simpler glucosides and almond-emulsin, not without an expectation that in such cases we should succeed in finding the β-glucase unaccompanied by amygdalase.

The use to which we had put leaves of the cherry laurel (*Prunus laurocerasus*)[†] had made this plant of special interest to us; on this account, we selected it, in the first place, for examination, more particularly because Hérissey had obtained from the leaves the cyanophoric compound prulaurasin,[‡] which Caldwell and Courtauld,[§] in the course of these studies, have shown to be a mixture of the two stereoisomeric glucosides of the formula $CN{\cdot}CHPh{-}O{-}C_6H_{11}O_5$, Fischer's glucoside and sambunigrin, the latter being the glucoside characteristic of the common black-fruited elder (*Sambucus nigra*).*

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[*] Fischer, 'Ber.,' 1895, vol. 28, p. 1508.

[†] 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 588.

[‡] 'Compt. Rend.,' 1905, vol. 141, p. 959.

[§] 'Chem. Soc. Trans.,' 1907, vol. 91, p. 671.

Finding that it was impossible to extract an enzyme from the comminuted leaf, we resorted to the expedient of using the finely divided leaf material and found that though this was practically without action on amygdalin, it readily effected the hydrolysis of Fischer's glucoside. Our conclusion was thus verified that two distinct enzymes are concerned in the hydrolysis of amygdalin and our hope realised that it would be possible to find one or other enzyme occurring alone.

It appears to be desirable to assign a distinct name in future to the enzyme in "emulsin" by which the resolution of the simple cyanophoric glucoside is effected; as it occurs very generally in the various species of Prunus, we propose to term it *Prunase;* also it will be convenient to use the name *Prunasin* in speaking of the glucoside (d-mandelonitrile glucoside) which, hitherto, we have termed Fischer's glucoside.

Prunasin has been isolated from the leaf of *Prunus padus*† and of *Prunus serotina*.‡ The leaf of the laurel, *P. laurocerasus,* as already mentioned, has afforded *Prunasin* together with the isomeric sambunigrin. In the case of elder also, sambunigrin is present in the leaf. Amygdalin, apparently, on the other hand, has never been separated from leaves but only from fruit kernels; the allied complex di-glucoside vicianin, which is an arabinose-glucose derivative, also occurs in the seed, not in the leaf.§

Apparently, the enzymes are distributed in a corresponding manner, prunase occurring both in leaf and fruit, amygdalase only in minute proportion at most in the leaf but in large proportion in the seed. A typical series of results illustrating the distribution of the two enzymes in the leaf and seed are given at the head of the table in the next communication (XVII, p. 363).

We venture to think that the argument put forward in previous communications is now fully justified and that it must be regarded as proved that "almond-emulsin" is a mixture of enyzmes two of which are successively concerned in the resolution of amygdalin. Failure to resolve amygdalin must be considered therefore to be primarily a proof that amygdalase is not present but in no way as an indication that the enzyme is absent which can only come into operation after amygdalase has acted. Evidence of the presence of this latter enzyme is afforded by the resolution of prunasin.

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* Bourquelot and Danjou, 'Compt. Rend.,' 1905, vol. 141, pp. 59 and 598.

† Hérissey, 'Arch. Pharm.,' 1907, vol. 245, p. 641.

‡ Power and Moore, 'Chem. Soc. Trans.,' 1909, vol. 95, p. 243.

§ Bertrand, 'Compt. Rend.,' 1906, vol. 143, pp. 832 and 970.

The introduction of prunasin, in place of amygdalin, as a test for prunase therefore constitutes an important advance in technique.* If amygdalin be used as a test material, assuming that it is hydrolysed only by the two enzymes amygdalase and prunase, the determination of the amount of hydrogen cyanide that is liberated serves as a means of estimating the combined activity of these two enzmyes; to ascertain if the former be present without the latter, it is necessary to ascertain whether glucose be set free.

But there is another possibility to be considered, since it has been shown by Giaja† that amygdalin may be resolved into a diglucose, hydrogen cyanide and benzaldehyde by means of the gastric juice of the snail. It is to be supposed that this enzyme occurs in plants, as Bertrand succeeded in converting vicianin into hydrogen cyanide, benzaldehyde and the arabino-glucose, vicianose, by means of an enzyme present in the seeds of *Vicia angustifolia*.

In view of the results now brought forward, it may be desirable to point out again‡ that the results obtained on using emulsin as a synthetic agent cannot be regarded as due to a single enzyme. We are engaged in making synthetic experiments with the simpler materials now at our disposal.

The high values given under prunasin in Table I (p. 364)—which are discussed in the next communication—are indicative inferentially of the presence of prunase, on the assumption that (in the absence of linase) prunase alone is able to hydrolyse prunasin. Until it has been shown that such is the case, they must be subject to this reservation.

Our desire is, we may say, to confine the term *Prunase* eventually to the specific enzyme which is the true correlate of *Prunasin*. It may be well to add that we apply the term β-glucase to enzymes generally that are able to resolve β-glucosides and are controlled by glucose—in other words, to enzymes which are adapted to the glucose section of the β-glucosides.

As the conclusions which we have formulated in earlier numbers of these studies are to some extent called in question by Bayliss in Note E, in the second edition of his monograph 'On the Nature of Enzyme Action,' we may refer briefly to his criticisms. The results now brought forward should remove some of his difficulties. Bayliss is inclined to question the doctrine that enzymes are strictly specific agents. In our opinion—and we have lived with them during so many years that we claim to have some feeling on the subject—the evidence is overwhelming that they are, especially as there is reason to believe that we always deal with a variety of enzymes when using natural products.

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* Compare 'Proc. Physiol. Soc.,' 1910, xxxiii; 'J. Physiol.,' vol. 40.

† 'Compt. Rend.,' 1910, vol. 150, p. 793.

‡ Compare No. XII, p. 323.

Bayliss appears to have been impressed by the contentions advanced in a lengthy degree dissertation published in the 'Zeitschrift für physikalische Chemie'* by Fajans, who bases his contentions on experiments which apparently have no connexion with the action of enzymes.

Bredig and Fajans have found that, on heating the two isomeric optically active *d* and *l* camphorcarboxylic acids with an optically active base, the one acid undergoes decomposition into camphor and carbon dioxide more readily than the other. It is assumed by Fajans that the optically active base functions as a catalyst—just as an enzyme does—and that whilst both acids are decomposed, the base acts preferentially, so that one acid is decomposed by it to a greater extent than the other acid. The assumption that in such a case the action is catalytic and comparable with that exercised by an enzyme is purely gratuitous, however.

Fajans himself admits that the salt is the substance that undergoes decomposition. As the salts formed from two corresponding optically active acids and an active base would not be optical opposites, the base, presumably, being differently situated in the two salts, would exercise a different attractive force in loosening carbon dioxide; it is not surprising, therefore, that the two salts undergo decomposition at different rates.

Fajans asserts that every order of difference is to be found in the behaviour of an enzyme towards optical isomers but this is definitely not true. A number of the cases to which he refers are not to the point—thus it is in no way established that the oxydases are enzymes and the fact that moulds are able to utilise both components of racemic amino-acids is easily accounted for in view of the ease with which these acids are racemised by ordinary agents. In the case of lipase, there is no reason to correlate the activity of the "enzyme" with optical activity—it is a selective agent only in the sense that it is a specific hydrolyst of ethereal salts of carboxylic acids. In point of fact, the only definition that can be given, at present, of an enzyme that will separate enzymes from other hydrolytic agents is one which involves the assumption that they act selectively.

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* 1910, vol. 73, pp. 25—96.

Studies on Enzyme Action. XVII.—*Enzymes of the Emulsin Type.* (II) *The Distribution of β-Enzymes in Plants.*

By H. E. Armstrong, F.R.S., E. F. Armstrong, and E. Horton.

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D 8014 Enzymes of the emulsin type.
D 6525 Detection and distribution of β-enzymes in plants.]

In extending our search for enzymes of the emulsin type, during the past two years, we have examined a large number of species of plants, using for the purpose the following method briefly referred to in the previous paper, as in most cases we found that active extracts could not be obtained directly from the leaf material.

Usually, as soon as possible after it has been collected, we have placed the plant in a bottle with toluene, both in order to prevent the development of moulds and to give full opportunity to the enzymes within the cells to resolve the various hydrolytes with which they are associated. Such material can be dealt with at leisure. It is first cut up in a mincing mill and then dried—either directly or after it has been thoroughly washed to deprive it of soluble matter—best by exposure in a vacuum desiccator at air temperatures; the washed material has the advantage that little or no correction has to be applied subsequently, in the course of the analytical process, on account of soluble matter, such as reducing sugars and tannins, which disturb the result, if present. But in most cases washing diminishes the activity of the material. In determining the original activity of the plant material, it is, of course, necessary to dry it without washing. The dried material is finally prepared for use by grinding it to a fine powder in a coffee mill. Such powder apparently may retain its activity over long periods.

We have used hitherto as hydrolytes the four glucosides: linamarin, amygdalin, prunasin and salicin, our primary object being to determine the distribution of the four enzymes: linase, amygdalase, prunase and salicase.

The method adopted has been to add the dried powder, prepared in the manner described, to a solution containing one-fifth of a molecular proportion of the glucoside per 1000 c.c., using solution and powder in the proportion of 100 c.c. to 1 grm. After digesting the mixture at 37°

Table I.

Plant.	Linamarin.	Amygdalin.	Prunasin.	Salicin.
Prunus laurocerasus (Oct., '10)	1·0	1·2	61·5	39·1
" " (Nov., '10)	1·2	1·5	40·7	21·0
" " seed	—	84·4	89·2	—
" " seed husk	—	1·5	9·7	—
" *amygdalus* (*dulcis*)	2·2	1·7	13·7	—
" " (*nana*)	1·2	1·5	17·1	6·6
* " " (*amara*)	—	—	10·5	3·6
Laurus lusitanica	0·7	3·1	45·7	47·2
" " seed	—	86·5	91·7	—
" " seed husk	—	10·7	19·5	—
Aucuba japonica	0·5	2·5	79·5	67·6
" " seed	—	3·0	76·0	56·7
" *longifolium*	0·5	3·2	76·5	48·7
Garrya elliptica	0·5	1·2	32·7	29·8
" *thuretii*	0·7	0·7	13·2	—
Laurustinus	0·5	0·5	1·2	—
Skimmia japonica seed	—	—	2·2	—
* *Salix rubra* (1910)	0·7	3·8	16·5	27·3
" " (1911)	—	—	—	45·7
Epilobium angustifolium ('10)	1·5	2·3	16·5	9·6
" " ('10)	0·7	0·7	4·1	4·8
Epilobium angustifolium—				
*Swiss, Saas Valley ('10)	—	—	5·7	29·1
Ayrshire ('11)	—	—	—	4·5
Myrdal ('11)	—	—	—	34·7
Norwegian Voss-Bergen ('11)	—	—	—	14·5
Fjaerland ('11)	—	—	—	17·1
Epilobium hirsutum (Thames) ('11)	—	—	—	3·2
Gaultheria shallon	0·5	0·7	1·6	0·7
" *procumbens*	—	—	26·0	10·0
" " seed	—	—	27·5	7·0
Arbutus unedo	0·5	0·7	4·2	1·8
Calluna vulgaris	—	—	3·0	—
* *Arctostaphylos uva-ursi*	—	—	1·0	6·0
* *Vaccinium Myrtillus*	—	—	29·0	1·0
" *uliginosum*	—	—	—	—
* *Castanea sativa* (spring, '10)	1·5	6·2	65·5	55·8
" " (autumn, '11)	—	—	—	—
Vicia sativa	1·2	1·6	11·5	—
" *cracca*	8·0	7·8	24·7	15·8
" *sepium*	3·2	4·5	36·9	31·8
" *sylvatica*	1·8	2·5	11·1	—
" *villosa*	2·5	2·5	10·0	—
Lathyrus pratensis	2·0	1·5	5·6	—
" *aphaca*	1·2	0·7	2·3	—
Ononis arvensis	—	—	28·0	—
Medicago sativa	6·7	3·2	14·5	—
Onobrychis sativa	2·5	2·0	7·5	—
Trifolium pratense	2·5	2·5	8·2	—
Galega officinalis	—	—	3·0	—
Lythrum salicaria	—	—	15·7	—
Spiræa ulmaria	—	—	4·0	—
Lotus corniculatus	64·5	2·7	32·0	27·8
" *uliginosus* (*major*)	1·8	1·5	2·0	—
" *jacobæus*	86·0	—	—	—
Aquilegia vulgaris	1·5	—	—	—
Thalictrum aquilegifolium	0·8	—	—	—
Asperula odorata	—	—	4·2	—
Galium verum	—	—	8·6	3·2
Isatis tinctoria	—	—	2·7	—
Rubia tinctoria	—	—	1·7	—

* In these cases the leaves were thoroughly washed before being dried and ground.

during 24 hours, the extent to which hydrolysis had been effected was determined by estimating the amount of hydrogen cyanide liberated from linamarin, amygdalin and prunasin and the amount of glucose liberated from salicin.

The results recorded in Table I were all obtained in the manner described. They afford opportunity for comment in many directions.

It was contended in No. XIII of these studies that the glucoside phaseolunatin present in *Phaseolus lunatus*, which is identical with that in flax (linamarin), is not, as Dunstan, Henry and Auld have asserted, an α-glucoside but a β-glucoside resembling prunasin; also that the accompanying enzyme phaseolunatase, which there is reason to believe is identical with the linase present in flax, is a β-enzyme of the emulsin type, though incapable of acting on amygdalin. These conclusions are entirely in accordance with the observations now recorded.

It should be mentioned that the linamarin we have used was extracted by ourselves from *Phaseolus lunatus* beans.

The values given in the first column of the table under linamarin are low with few exceptions, the most striking being that afforded by *Lotus corniculatus*. As this plant has been fully discussed in a previous communication,* little need be said of it now beyond pointing out that it also has a relatively high degree of activity towards prunasin and salicin such as is not met with in other cases; it is evident that *Lotus corniculatus* contains more than one β-enzyme and in view of the results recorded in the communication referred to it will be important to ascertain whether these enzymes vary concomitantly or independently.

It is open to question whether values below 1 to 2 per cent. are of any significance. It is possible that such small amounts of action—if not mere experimental errors—are due to the presence of small amounts of a cytase; but the distinctly high values obtained in the case of *Vicia cracca* and *Medicago sativa* are doubtless significant, particularly as there is an obvious tendency towards high values in the case of nearly all the leguminous plants examined.

Judging from the values in the Amygdalin column, amygdalase—the enzyme by which the first molecule of glucose is removed from amygdalin—is but sparsely distributed and almost confined to seeds in which amygdalin is present. It is in no way certain that the low values in this column represent the activity of amygdalase proper; indeed, it is specially significant that high values appear in the case of Vicia species, in view of the occurrence of

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* 'Roy. Soc. Proc.,' 1912, B, vol. 84, p. 471.

Vicianin in *V. angustifolia*[*] together with an enzyme (vicianase) by which it is resolved into the arabino-glucose vicianose, benzaldehyde and hydrogen cyanide. It may well be that the high values in the amygdalin column are in some cases to be taken as indications of the presence of vicianase.

It should be again pointed out, now that it is established that two enzymes are concerned in the resolution of amygdalin, that failure in obtaining hydrogen cyanide from amygdalin is proof only of the absence of amygdalase (perhaps also of vicianase), not of that of an enzyme capable of liberating hydrogen cyanide from a cyanophoric glucoside; amygdalin, as before stated, is not the proper test material to use in discovering enzymes of this latter class.

The values recorded under Prunasin are high in a large proportion of cases, showing that prunase (or a closely allied enzyme) is widely distributed.

It is more difficult to interpret the results obtained with salicin in comparison with prunasin. Sigmund[†] has contended that the enzyme present in Salix is not "emulsin" because it has no action on amygdalin; such behaviour, however, as pointed out above, is only proof of the absence of amygdalase. But it will be noticed that whereas, in most cases, prunasin is more attacked than Salicin, the reverse is true of *Salix rubra* and of an *Epilobium angustifolium* of Swiss origin, the latter having proved to be five times as active towards salicin as it was towards prunasin. It would seem probable, on the whole, that prunase is less active towards salicin than towards prunasin; also that a distinct enzyme, salicase, exists, which is only capable of acting on salicin perhaps and that the two enzymes occur together in some cases.

The glucosides apparently may undergo hydrolysis in two ways, according as they are attacked from the side of the glucose group or from that of the radicle associated therewith. The importance of this conception has already been demonstrated in the case of lactose (compare No. XII, 324). As prunase is controlled by glucose, there is good reason to suppose that this enzyme becomes attached to the glucose section of the molecule and that this is the reason why it is able to determine the hydrolysis of so large a proportion of the known β-glucosides. The enzymes occurring together with many of the glucosides may owe their specific character to the fact that they are compatible not with the glucose group in the glucoside but with the radicle which is associated with it. Salicase, from this point of view, is perhaps an enzyme which acts on salicin through the agency of the saligenin radicle. On this account, it is interesting to note that *Gaultheria procumbens* has a relatively slight action on salicin, though it contains a glucoside, gaultherin, which is closely related to salicin, thus:—

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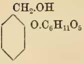
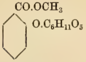
[*] Bertrand, 'Compt. Rend.,' 1906, vol. 143, p. 832.

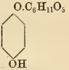
[†] 'Monatsch.,' 1909, vol. 30, p. 77.



Salicin.



Gaultherin.



Arbutin.

The slight activity towards prunasin of *Asperula odorata*, *Galium verum*, *Isatis tinctoria* and *Rubia tinctoria* is explicable from this point of view, as the enzymes which these plants contain are probably active only towards the glucosides by which they are severally characterised—coumarin-glucoside indican and ruberythrinic acid.

On the other hand, it is conceivable that a difference such as is here referred to may be sufficient to affect an enzyme compatible with the glucose group in the glucoside and also immediately compatible with the group associated with glucose in a particular glucoside. Thus the group $CO.OCH_3$ in gaultherin may be sufficiently different from the group $CH_2.OH$ in salicin to make it impossible that the enzyme naturally associated with the one compound should be able to enter into such relationship with the other compound that it can promote its hydrolysis.

The following values obtained with arbutin are of interest from this point of view :—

	Percentage hydrolysed.
Arctostaphylos uva-ursi (bearberry)	2·5
Arbutus unedo	0
Epilobium angustifolium (Swiss)	22·5
Salix rubra (1911)	13·7

It may be, of course, that in some cases, the material at our disposal has not been gathered at the right season and has contained little or no enzyme. To make the work complete the influence of season must be taken into account.

Obviously, a vast amount remains to be done before these and other similar problems have been solved. When suitable materials have been discovered, it will be necessary to institute a close comparative study of the action of the enzymes on a variety of materials. Sufficient has been done to show that the method is one that can be applied generally.

Thus far our remarks have been directed mainly to the evidence the results afford as to the character of the enzymes present in various plants. There are other points to which it is desirable to refer briefly in conclusion.

The results quoted are in no way representative of *Prunus laurocerasus;* younger leaves are more active towards prunasin. We are making a special study of this leaf with a view to determining the variations to which it is subject during the entire season. At all times it contains a very high proportion of cyanophoric glucoside; the absence of amygdalase and of amygdalin from the leaf is therefore all the more remarkable in view of their presence in the seed.

Most of the varieties of *Prunus* we have been able to test afford hydrogen cyanide but the amount procurable from the leaf is often very small and is subject apparently to great variation throughout the season. At times it is scarcely detectable in leaves of the bitter almond and these often contain less than do those of the sweet variety growing alongside them. In the case of the fruit, cyanide is present during the earlier stages even in the sweet almond but disappears as it ripens.

We have never obtained more than minute amounts of hydrogen cyanide from leaves of *Laurus lusitanica* and have often failed to detect any; it is present in minute proportion in the flower and usually in young leaves formed at the flowering period and soon afterwards. The leaf is intensely bitter; this fact, taken together with the high activity towards salicin is probably an indication of the presence of a glucoside other than prunasin.

We were fortunate in securing a considerable quantity of the ripe fruit last autumn and from this were able to separate amygdalin without difficulty merely by extraction with boiling alcohol.

On analysing the seed kernels, they were found to contain: oil, 9 per cent. (iodine number, 90·4); protein, 26·9 per cent.; moisture, 4·1 per cent.; ash, 2·8 per cent.

The *Aucuba japonica* leaf is rich in enzyme and judging from the variation in the values obtained with prunasin and salicin it is possible that more than one is present. The absence of amygdalin from the seeds, coupled with the fact that these contain a large percentage of the glucoside aucubin, is confirmation of our view that the formation of amygdalin in the Prunus fruit is consequent on the presence of amygdalase. The two species of *Garrya* examined are allied to *Aucuba* but less rich in glucoside (aucubin); it will be noticed that they also contain less enzyme.

The variations in activity towards salicin shown by the several specimens of *Epilobium angustifolium* we have examined are remarkable. That of Swiss origin was picked in the Saas valley in August 1910 and we were specially attracted by its sturdy growth and willow-like character; this in fact led us to test its action on salicin. Two separate specimens from the Midlands obtained in the following September were far less active and a specimen picked in an Ayrshire garden at Whitsuntide, 1911, was similar to these. The specimens secured in August last year in three different districts in Norway correspond fairly with the Swiss specimen in activity, that picked at a considerable elevation in the Myrdal resembling the Swiss plant most closely.

The absence of "prunase" from the *Ericaceæ* examined, except in the case of the Whortleberry, is of interest in view of the occurrence of Arbutin in this group.

The great activity of Spanish Chestnut towards prunasin and salicin is significant; apparently no glucoside characteristic of this species is known.

Another instance of variability is afforded by *Aquilegia.* Apparently hydrogen cyanide is to be detected in many if not in most species of this family but the amount is subject to considerable variation at different seasons and as often as not it cannot be detected in cultivated garden varieties of the plant.

Mountain Ash (*Pyrus aucuparia*) again varies greatly in the amount of hydrogen cyanide the leaf affords at various seasons; often it cannot be detected.

A noteworthy instance of association in habits is presented by *Thalictrum aquilegifolium,* the leaf of which closely resembles that of aquilegia. We have tested this plant in many places and always found cyanide present in the leaf; judging from tests made at Kew, other species do not contain the cyanide.

The Vicia group presents interesting peculiarities in addition to those indicated in the table. The seed of *Vicia angustifolia* contains a considerable proportion of the cyanophoric glucoside Vicianin, which Bertrand has also found in two other species,* though only in small proportion. On more than one occasion we have obtained traces of hydrogen cyanide from unripe seeds of *V. sativa. V. villosa,* in like manner, affords traces of the cyanide but only at the moment when the pod blackens; none can be obtained from the ripe seeds. Whether corresponding variations take place in the enzymes remains to be ascertained; results such as these, however, are interesting proof of latent peculiarities.

We hope to be able to submit the cases mentioned to more exhaustive systematic study on lines similar to those followed in the case of *Lotus corniculatus.* It is to be expected that, when completed, such observations will afford important evidence as to the possibility of differentiating botanical species by chemical means and with reference to the transmission and variation of chemical factors, such as enzymes and glucosides, in plants. The evidence now brought forward affords renewed proof of the essentially selective nature of the actions exercised by enzymes. By pursuing the inquiry on the lines indicated and by using a larger number of hydrolytes, we hope to be able eventually to decide both the specific character and the range of activity of the various β-glucases—but it is obvious that the task will be one of extreme difficulty.

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* 'Compt. Rend.,' vol. 143, p. 970.

Studies on Enzyme Action. XVIII.—*Enzymes of the Emulsin Type.* (III) *Linase and other Enzymes in Linaceæ.*

By H. E. Armstrong, F.R.S., and J. Vargas Eyre, M.A., Ph.D.

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D 8014 Enzymes of the emulsin type—linase.
D 8030 Variations of glucoside and enzyme in linseed.
K 3120 } Enzymes in Linaceæ.]
D 6525 }

In No. XIII of these studies, communicated to the Society early in 1910, a long series of observations was recorded from which the conclusion was deduced that the glucoside extracted from the seeds of the wild variety of *Phaseolus lunatus* and the enzyme associated with the glucoside both belong to the β-series, a conclusion not in harmony with that arrived at previously by Dunstan, Henry and Auld.*

The glucoside termed by Dunstan and Henry *Phaseolunatin* was first isolated from young flax plants in 1891 by Jorissen and Hairs,† by whom it was named *Linamarin*. Jorissen had previously found that "emulsin" had no action on the cyanophoric constituent of flax, though the glucoside was readily attacked by the enzyme occurring in the seed.

Jorissen's discovery was confirmed in 1903 by Dunstan, Henry and Auld. The flax enzyme, according to their statement, has a range of activities similar to that of almond-emulsin, readily hydrolysing amygdalin and salicin.‡ There is nothing to show, however, that their conclusion was based on quantitative determinations. On account of this statement and on various other grounds, it appeared to us that it was desirable to study the "enzyme" in flax more thoroughly: one reason in particular was our desire to discover a material from which we could obtain the enzyme whenever necessary, for assuming that the two plants yield the same enzyme, as linseed is so easily procured, it obviously is a far more convenient source of the enzyme than is *Phaseolus lunatus*, a seed which is no longer on sale in this country.

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* 'Roy. Soc. Proc.,' 1906, B, vol. 78, p. 145 ; 1907, B, vol. 79, p. 315.

† 'Bull. Acad. Roy. Belg.,' 1891, vol. 21, p. 529.

‡ It is asserted by Dunstan, Henry and Auld that—"In subsequent papers, Jorissen stated that both *Linum usitatissimum* and *Linum perenne* contain amygdalin in the leaves and stems." This is incorrect. Jorissen speaks of "an amygdalin," not of actual amygdalin, using the term in a generic sense as meaning a substance which behaves like amygdalin on hydrolysis.

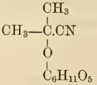
The observations we were able to make with several of the *Linaceæ* during the season of 1910 were of such interest in connexion with our inquiry into the nature and distribution of enzymes and in other respects also, that we were led to extend the inquiry to the family generally during the past year; we, therefore, grew all the species we could procure; this work was carried on simultaneously with that recorded in the two previous communications and that on *Lotus corniculatus.*

We propose in future to speak of the specific enzyme corresponding to linamarin as *linase,* as it appears to be present in so large a number of species of *Linaceæ* as to be characteristic of the genus.

A question of primary importance to be answered was whether the properties deduced from the study of the effects produced by "*Phaseolunatase*" were those characteristic of a single enzyme *linase* or due to a mixture of enzymes.

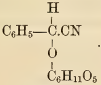
Armstrong and Horton, in agreement with Dunstan and his co-workers, came to the conclusion that the "enzyme" extracted from *Phaseolus lunatus* is practically without action on amygdalin; they found, however, that it acted readily on prunasin as well as on linamarin (phaseolunatin). Now that it is established that "emulsin" is a mixture of several enzymes, one of which resolves amygdalin into glucose and prunasin, it is obviously easy to explain the difference between the behaviour of the Phaseolus enzyme and that of emulsin—to realise, in fact, that it is due to the absence of amygdalase from the Phaseolus preparation. But whilst both linamarin and prunasin are hydrolysed by the Phaseolus "enzyme," prunase, the correlate of prunasin—whether it be prepared from the almond or (as shown in the previous communication) from any other source—is without action on linamarin. It is important that the significance of this difference should be fully understood.

The two glucosides are represented by the following formulæ:—



$$\begin{array}{rl} & CH_3 \\ & | \\ CH_3- & C.CN \\ & | \\ & O \\ & | \\ & C_6H_{11}O_5 \end{array}$$

Linamarin.



$$\begin{array}{rl} & H \\ & | \\ C_6H_5- & C.CN \\ & | \\ & O \\ & | \\ & C_6H_{11}O_5 \end{array}$$

Prunasin.

The recognition of two distinct enzymes, each corresponding to one of these two compounds, the one (linase) capable of determining the hydrolysis of both glucosides, the other (prunase) limited in its action to the single glucoside to which it corresponds, would be a fact of no slight consequence in connexion with the theory of enzyme action.

The conclusion that both glucosides can be hydrolysed by one enzyme (linase) rests, however, at present, on the observations made with the enzyme or enzymes extracted from the Phaseolus bean; hence the importance of ascertaining the effect produced by enzymes obtained from other sources. Whatever their range of activity, as the activity of both enzymes is much diminished by the presence of glucose, it is to be supposed that both are so constructed that each can fit the glucose section of its "co-glucoside" while in correspondence also with the radicle with which the glucose residue is in association; so that if it be proved eventually that only the enzyme corresponding to the simpler compound is compatible with both glucosides, it will follow that the smaller key can be passed into both locks but that the larger will not fit into that to which the smaller key properly belongs. The problem whether linase can exercise such a dual activity, therefore, is one of very great interest.

Hydrogen Cyanide from Linaceæ.

Of late years, much uneasiness has been felt in agricultural circles owing to statements that have been made that hydrogen cyanide may be present even to a dangerous extent in linseed cake. There is no doubt that usually, if not always, it can be detected in the commercial article. In view of the constant presence of linamarin in the green plant and the unripe seed, this is not surprising. During the past two years we have studied the seed systematically from this point of view.

It is easy to detect hydrogen cyanide even in a single flax seed by means of Guignard's picrate test. For this purpose, a convenient length—2 or 3 inches—of narrow quill tubing is sealed at one end and rounded in the flame at the other; a similar piece of narrower tube, which will just slide into the wider tube, is also prepared; and an air-tight joint between the two tubes is provided by means of a short length of narrow rubber tubing. After the seed to be tested has been pricked in several places with a pin, it is placed at the bottom of the larger tube together with a drop of water and a minute drop of chloroform. A diamond-shaped piece of moist picrate paper, a few square millimetres in area, having been fixed in the open end of the narrower tube, this tube is adjusted within the wider tube so that the picrate paper is just above the seed. The tube is then maintained at about 35° in an incubator or simply kept in the waistcoat breast pocket. If cyanide be present, the yellow paper darkens and becomes orange or orange-red within 24—48 hours.

We had fairly satisfied ourselves during 1910 that the presence or absence of the cyanide was dependent on the degree of maturity of the seed; we were able, during the past season, which was so exceptionally favourable to ripening, to place this conclusion beyond doubt. Ripe seeds were always found to be free from cyanide but we have invariably detected it in unripe seeds. Flax being a plant which continues, during a considerable period, to flower and produce seed, the seeds never ripen all at once, so that when the crop is harvested the plants always carry a mixture of ripe and unripe seed: it is therefore easy to understand the presence of cyanide in the commercial cake.

The tendency has been to regard the potential presence of hydrogen cyanide in linseed cake as harmful but taking the very special and peculiar properties of the cyanide into account, it may well be that it is of positive condimental value and that the peculiar value of linseed cake as a cattle food is at least in part due to the liberation of minute proportions of such a substance.*

Hydrogen cyanide was detected by Jorissen in only two of the Linaceæ, *L. usitatissimum* and *L. perenne*. During 1910, owing to the kindness of Dr. Hugo Müller, we were able to test several of the yellow flowered species, in addition. Last year, we procured seed from various European Botanic gardens and from seedsmen of as many varieties of Linaceæ as possible and grew these ourselves. A considerable proportion of the seeds we obtained bore fancy names and proved to be varieties either of *L. usitatissimum* or of *L. angustifolium* or *L. perenne*. We were able to satisfy ourselves that all the species resembling either ordinary flax (*L. usitatissimum*) or *L. perenne* in general habit of growth, carrying white, blue or red (*L. grandiflorum*) flowers, were more or less richly cyanophoric; we uniformly failed, however, in obtaining hydrogen cyanide from the yellow-flowered species (*L. arboreum*, *L. flavum*, etc.). The amount of cyanophoric glucoside present in different species is different and is subject to variation throughout the period of growth. In the case of *L. usitatissimum*, the maximum proportion is reached at a very early stage but even when mature and full of seed this species still contains cyanide. In the case of *L. perenne*, the mature foliage may contain no cyanide, although it is easily detected in the young shoots. *Linum grandiflorum* appears to contain the major proportion of cyanide at the flowering period.†

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* Compare 'Roy. Soc. Proc.,' 1912, B, vol. 84, p. 471.

† *Note added June* 24, 1912.—The observations have now been extended to other species of blue-flowering Linaceæ, and evidence has been obtained that besides *L. perenne* several others, namely *L. narbonense*, *L. hologynum*, and *L. suffruticosum*, also lose the cyanophoric glucoside at quite an early stage of their development.

Up to the present the following yellow-flowering species have been examined:—*L. arboreum*, *L. campanulatum*, *L. flavum*, *L. flavum luteum*, *L. salsoloides*, *L. maritimum*, A very extended series of observations must be undertaken if the manner in which variation in the amount of cyanide takes place throughout the period of growth in various species is to be fully elucidated: the inquiry is in progress.

One of the authors has had the opportunity during the past summer of studying the growth of flax in various districts in Europe where it is systematically cultivated and has thus been able to gather much information of a suggestive character. Many who have carefully studied the growth of flax from a commercial standpoint freely express the belief that flax is a plant which rapidly becomes adapted to new conditions of soil and climate—they say it degenerates. There are instances of seed taken from a blue-flowering crop giving a crop of flax bearing white flowers when raised under different conditions of climate; in this connexion it is of interest to observe that when seed from uniformly blue-flowered flax, grown in the Baltic Provinces, is grown in Holland, Belgium or some more southern country, it always gives a crop containing numerous white-flowering plants in the first year; on the other hand, white-flowering flax is not stable in the more central parts of Russia, passing almost completely into the blue form in about four seasons.

It is often stated, on good authority, that the characteristics of good fibre-flax become lost to some considerable degree when the plant is repeatedly grown in Holland or Belgium; a healthy crop of tall, straight-stemmed flax becoming less tall, coarser in stem and generally inferior, besides being less able to resist disease after growing during four or five years in succession. Again, in South Russia, a form of flax is grown, known as "Steppe-seed" flax, which exhibits a greater tendency to branch and to carry seed and does not attain the height of the flax grown in the North for the production of fibre. When "Steppe-seed" is taken north and grown in the cooler and moist regions, the plant gradually changes its character and, after four or five years' acclimatisation, the crop raised from such seed is quite as tall as the usual fibre crops and the fibre it yields is equal to that usually produced in the district. In view of this kind of information and the somewhat obscure relationship between the various forms, it will be of interest to compare carefully the different varieties of *Linum usitatissimum* from various points of view.

It is thought in the Baltic provinces that the fibre is spoilt if the seed be

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and *L. gallicum* (?); no trace of hydrogen cyanide has been detected either in the young or in the mature foliage. It is possible that small quantities of linamarin and its "co-enzyme" are present at some very early stage but if so the period must be of short duration.

allowed to ripen upon the plant—the nature is said to be drained out of it; the tops carrying the seed are therefore cut off and allowed to ripen separately by exposure to the sun on rough drying frames. The seed is often afterwards put into a heated chamber to advance the ripening and drying, so that it may be put upon the market.

The accumulation of linamarin in the seed takes place at a comparatively late stage, though the glucoside disappears eventually. The very young seed pod affords only a faint indication of hydrogen cyanide; the largest amount is obtained when the seed assumes a full green colour. There is a coincident increase in the proportion of enzyme, as shown by the following results obtained by digesting 1 grm. of the entire seed capsule with a solution of linamarin:—

Capsules	Very small.	Medium size.	Fully grown.
Percentage of activity	0·8	1·25	9·75

The Enzymic Activity of the Linaceæ.

We have applied the method developed in the two previous communications to the study of the enzymes present in the leaf and seed of various species of Linaceæ, and have dealt with more than 60 reputed species.

It is a striking fact that apparently, in this genus as in that of *Lotus*, enzymic activity is correlated with the presence of a cyanophoric glucoside; the yellow-flowered species, which are free from cyanide, show little if any enzymic activity towards Linamarin, amygdalin, prunasin and salicin. The fact is, perhaps, not without significance that these species are all slow in germinating and slow in growth.

Some of the results obtained with the various species, showing the enzymic activity of leaf and seed, are recorded in Tables I and II; the values given in Table II are calculated from those given in Table I, supposing the degree of activity towards Linamarin to be the same in all cases.

The results obviously require interpretation from various points of view. It is clear, in the first place, that a variety of enzymes are present in the leaf and seed of the various species and that the proportions in which they are present are subject to considerable variation.

The activity towards linamarin may safely be attributed to linase and linase alone. *L. catharticum* is the only one of the species examined which has a low degree of enzymic activity and almost no action on linamarin; this species also affords but a small proportion of hydrogen cyanide.

Table I.

Material examined.	Leaf.				Seed.			
Linaceæ.	Glucosides.				Glucosides.			
	Lin.	Amy.	Prun.	Sal.	Lin.	Amy.	Prun.	Sal.
L. grandiflorum rubrum ...	75·7	4·7	80·2	63·3	67·5	24·5	41·5	20·4
L. grandiflorum roseum ...	—	—	—	—	69·8	21·2	36·2	—
L. usitatissimum	80·7	3·7	36·7	33·0	73·1	10·0	33·5	31·7
L. usitatissimum album ...	78·5	3·3	67·7	34·7	71·5	7·2	39·7	35·0
L. alpinum	77·7	3·0	58·2	—	—	—	—	—
L. lewisii (perenne).........	51·2	4·5	21·2	—	—	—	—	—
L. catharticum	1·5	0·8	13·5	8·0	—	—	—	—
L. perenne	—	—	—	—	11·7	2·8	8·7	—
L. perenne album	87·0	5·5	33·3	—	17·2	5·2	12·5	—

Table II.

Material examined.	Leaf.				Seed.			
Linaceæ.	Glucosides.				Glucosides.			
	Lin.	Amy.	Prun.	Sal.	Lin.	Amy.	Prun.	Sal.
L. grandiflorum rubrum ...	80·7	5·0	85·5	67·5	80·7	29·2	49·6	24·3
L. grandiflorum roseum ...	—	—	—	—	80·7	24·5	41·8	—
L. usitatissimum	80·7	3·7	36·7	33·0	80·7	11·0	36·9	35·0
L. usitatissimum album ...	80·7	3·4	69·6	44·4	80·7	8·1	44·8	39·5
L. perenne	—	—	—	—	80·7	19·8	60·0	—
L. perenne album	—	—	—	—	80·7	24·4	58·6	—
L. catharticum	80·7	43·0	726·3	430·0	—	—	—	—

The results recorded in No. XIII of these studies show that the "enzyme" of *Phaseolus lunatus* is about equally active towards linamarin and prunasin. As already pointed out, it in no way follows from this result that linase is equally active towards these two glucosides; the hydrolysis of the prunasin may have been due in part, if not entirely, to prunase being present together with linase. It is clear that some such reservation must be made in view of the results given in the above tables: in only one case (*L. grandiflorum*) is the activity towards linamarin and prunasin equal; in half a dozen, the prunase value was about half the magnitude of the linase value, showing that linase is not equally active towards the two glucosides and that—as is to be expected—it is more active towards the correlated glucoside.

It is clear that "prunase" accompanies linase both in *Phaseolus lunatus* and in many *Linaceæ* and from this point of view the high prunase value given by *L. catharticum* is of interest. It may well happen that linase *per se* will be found to be without action on prunasin—such a conclusion would be justified, if a case could be found in which the values given under linamarin and prunasin for *L. catharticum* were reversed.

The problem is similar to that presented by "emulsin," which in the course of these studies has been proved to contain at least two distinct enzymes, amygdalase and prunase, both of which are concerned in the resolution of amygdalin. Prunase having been found without linase in many plants, the problem now before us is, if possible, to find linase without prunase.

These arguments will at least serve to indicate the importance of the inquiry on which we are engaged and the value of the method which has been devised of studying plant enzymes comparatively.

To consider the other materials hydrolysed, the amount of "amygdalase" in the leaf material is evidently small but in the seed, in the case of *L. grandiflorum*, the amount indicated is surprisingly large, particularly when the low values given under prunasin are considered.

But evidently much depends on the period of growth, as shown by the following values (percentage activities) given by *L. grandiflorum rubrum* (leaf material) in June and September, 1911 :—

	Linamarin.	Prunasin.	Amygdalin.
June	86	74	1·7
September	79	80	4·7

It is noteworthy (Table I) that whilst the prunasin values obtained with leaf materials are all very high relatively to the "amygdalin" values, in the seed there is not the same disproportion between them, the amygdalin values increasing apparently at the expense of the prunasin values. As amygdalin is not present in the seed, it does not seem probable that the high amygdalin values are due to the presence of amygdalase; a more probable explanation perhaps is that vicianase or an allied enzyme is present, especially in the seed.

The salicin values appear to bear no direct relationship to the linamarin values and are always below the prunasin values though often very near to these latter. It is a question whether linase has any action on salicin. Prunase apparently has the power of hydrolysing salicin and as the prunasin values are never lower than the salicin values but often in excess of them, it is not improbable that salicin is less readily hydrolysed by prunase than is prunasin and that some other enzyme other than prunase, capable of hydrolysing prunasin but not salicin, may be present at times together with prunase. This conclusion is perhaps more in harmony with that indicated above that vicianase may be present together with linase.

Such briefly are the problems before us. It may be that the differences observed are in some cases due to faulty determinations and much remains to be done in establishing valid methods of determining enzymic activity. But differences such as we have referred to are met with so constantly that it is clear that they correspond to actual variations in character and proportion of the enzymes present in plants.

Observations on Fowls and Ducks in Uganda with Relation to Trypanosoma gallinarum *and* T. gambiense.

By Dr. H. L. Duke. (With a Note by Miss Muriel Robertson.)

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received May 9,—Read June 6, 1912.)

[Plate 9.]

In the course of a considerable number of experiments, carried out to investigate the duration of infectivity of antelopes for *T. gambiense*, Uganda fowls were employed to nourish the flies during the earlier days of the experiments. The birds have been shown by the Commission of 1908–10 to harbour *T. gallinarum* in a certain number of instances, although nothing was known concerning the intermediate host of this trypanosome.

In the course of routine dissections of laboratory-bred flies from these experiments a curious crithidial type of flagellate was noticed in the hinder part of the mid-gut, similar to that reported recently from this laboratory by Captain A. D. Fraser and myself. These flagellates must have been derived either from the antelope upon which the flies originally fed or from the cock. In morphology and movement they were distinguishable at once from developmental stages of *T. gambiense* in *Glossina palpalis*. As some of the laboratory antelope had been found to be infected with *T. ingens* it appeared possible that the flagellates might represent developmental stages of this trypanosome. On the other hand suspicion fell upon *T. gallinarum*, although at the time microscopical examination of the fowls used had always proved negative.

To solve the point clean laboratory-bred flies were fed upon the suspected cocks, with the result that after two failures the crithidiæ were obtained in

Table I.

Expt. No.	Number of days flies fed upon cock.	Expt. No. of cock.	Examination of cock's blood for *T. gallinarum*.	Flies showing crithidiæ.	Day after first feed on cock.	Number of flies.		
						1st day.	30th day.	Dissected.
158	17	110	+	0	18	56	52	56
227	21	210	+	1		87	70	87
214	27	110	+	0		55	52	55
			Totals	1		198	174	198

Remarks.—The flagellates were present in the hinder part of the mid-gut only, and in large numbers.

Table II.

Expt. No.	Number of days fed on cock.	Expt. No. of cock.	Examination of cock's blood for *T. gallinarum*.	Flies showing crithidiæ.	Day after first feed on cock.	Number of flies.			Cage originally fed upon.
						1st day.	30th day.	Dissected.	
93	12	110	+	1	14	54	43	54	Reedbuck 2431.
383	26	384	+	1	54	112	98	112	Monkey 199 (*T. gamb.*)
394	17	431	—	3	14, 35, 49	91	66	88	,, 392 (arsenic)
428	12	462	—	1	4	119	66	118	,, 199 (*T. gamb.*)
429	11	465	—	1	19	89	63	89	,, 199 ,,
453	17	460	+	1	27	101	75	100	,, 401 ,,
			Totals	8		566	411	561	

Remarks.—The positive fly of Expt. 453 showed an infection of the proventriculus. In all other infected flies, flagellates were only present in the hinder region of the mid-gut.

the fly. The connection between *T. gallinarum* and these crithidiæ was further established on several occasions by the subsequent discovery of the trypanosomes on repeated examination of the cock's blood. Owing to the extreme difficulty of making certain that any given fowl was not infected with this trypanosome, it was not found practicable to conduct any systematic experiments on its development in *G. palpalis*. The actual day of development of any given infection cannot be established, as in every case the flies were a considerable time on the infected cock.

Although the blood of all the fowls employed was examined on numerous occasions, *T. gallinarum* was not always to be found in those birds which were proved by fly-dissection to be infected. The trypanosomes are very rare in the peripheral circulation, and even on centrifugalisation of considerable quantities of blood after death were only obtained in very small numbers.

The preceding tables show the cycle and other experiments in which these crithidiæ were seen in the course of the fly-dissection. In Table I the flies were fed upon infected cocks only. In Table II the flagellates were found incidentally in the course of cycle-experiments with *T. gambiense*.

The following Description of the Morphology of the Flagellates has been most kindly drawn up for me by Miss Robertson, to whom all positive flies were handed over :—

"In many respects the appearance of *T. gallinarum* in *G. palpalis* is exceedingly characteristic. The infection is rarely very numerous, seldom assuming anything like the virulence of *T. gambiense* or *T. nanum* in the same situation.

"The trypanosomes are, as a rule, situated pretty far back in the alimentary tract. The mid-gut of the fly, that is to say, the region between the proventriculus and the origin of the Malpighian tubules may conveniently be divided into three portions, anterior, middle, and posterior. The infection very rarely proceeds further forward than the middle portion. On one occasion only were these trypanosomes found in the anterior portion and in the proventriculus.

"The motion of the flagellate in the live state is very striking, and at once arrests the attention of any observer accustomed to the appearance of gut-forms of mammalian trypanosomes. The body is in the vast majority of the forms quite stiff through about two-thirds of its length, and is often broad and relatively massive, though the breadth is subject to variation. The flagellum is much thicker than in mammalian trypanosomes, and has a lashing motion, which drags the organism forward with a rapid movement of translation. This progression takes place in straight lines, and in the more slender drawn-out individuals may occasionally be reversed in direction.

"As it is impossible to tell when the flies first received the infecting trypanosomes, I have no information as to the exact sequence of forms in the cycle. The main outlines are, nevertheless, pretty obvious, and the massive broad forms gradually give place to smaller and more slender crithidial individuals, some of which, in what appears to be the later flies, show a considerable lengthening out of the body.

"As will be seen from the drawings (Plate 9), true trypanosome-phases have not been observed. Multiple division takes place, and is not by any means a very rare occurrence, although here, as in most trypanosome-cycles binary fission is the usual method of multiplication (see figs. 1, 2, 4, and 9). The process in question is rather irregular, and, after carefully studying a considerable number of these appearances, it becomes clear that it is a mere telescoping of successive binary divisions.

"The flagellate contains in the earlier infections a relatively large amount of dense protoplasm, and we have thus an illustration of the common biological process, whereby, in a cell encumbered with much protoplasm, the nuclear apparatus may undergo several successive divisions before the correlated splitting of the cell-body is accomplished.

"*Morphology.*—The films were fixed wet, by dropping them face downwards on to Schaudinn's fluid. The stains used were iron-hæmatoxylin, after Heidenhain's method, and hæmalum.

"The protoplasm, especially in the broader forms, is very dense and granular, a feature which is somewhat less marked in the later stages, Unfortunately, this density of the protoplasm obscures the final cytological details to a greater or less extent.

"The trophonucleus is of a common trypanosome type, consisting of a large central karyosome surrounded by a clear space, which is bounded in turn by a delicate membrane; fine rays pass from the karyosome to the membrane.

"The kinetonucleus (centrosome of French and blepharoplast of German writers) lies close to and generally in front of the trophonucleus. It is relatively large, and presents the somewhat curious double appearance of two closely-apposed granules lying one behind the other. This does not seem to be in any way a case of precocious division.

"In fig. 4, where division is actually in process, and where the two kinetonuclei are still joined by the centrodesmose, they nevertheless preserve the double appearance, and, in a case of multiple fission figured (fig. 9), the same thing is to be observed. Moreover, the direction of the plane of division is at right angles to the split in the kinetonucleus. In some of the latest stages of the material, namely, those from the proventriculus, this is less apparent, and the kinetonucleus seems more compact; still, even here the double nature of the structure can still be detected in most cases.

"The blepharoplast proper, *i.e.* the minute granule actually at the origin of the flagellum, is usually obscured, but can be seen in favourable specimens.

"Another structure not usually to be observed in trypanosomes, but which I have seen in the development stages of *T. raiæ*, a massive trypanosome not unlike *T. gallinarum* in appearance, is a granule situated towards the posterior, *i.e.* antiflagellar, end of the cell. This granule is not particularly small in size, but its outline is rarely very sharply defined; in some instances it appears to be attached by a fine line to the nucleus or to the blepharoplast. In some individuals there are two lines visible, one of which passes to the blepharoplast, the other to the nucleus. Owing to the density of the protoplasm these lines are not very clearly visible, and can be observed only in the more favourable specimens.

"The granule, on the other hand, is a very fairly constant appearance, and divides at divisions (figs. 1, 2, 4, and 9). In *T. raiæ*, in the developmental stages in the leech, these appearances are much clearer and more perfectly defined. In *T. gallinarum* the granule is not to be seen in the more slender stages.

"Figs. 1, 2, and 4 give an illustration of the more usual binary fission, the spindle of the trophonucleus shows the characteristic centrodesmose, but the finer details are never particularly clear.

"In conclusion, I may say that, while I have not so far met with a mixed infection, it appears that there would not be much likelihood of these forms passing undetected; nevertheless, crithidial individuals appearing in a cycle of a mammalian trypanosome, where cocks' blood had formed part of the food of the fly, would have to be rejected until confirmed from flies fed exclusively upon clean animals.

"*Note.*—From the nature of the above infections, it appears that *Glossina palpalis* is certainly not the normal host of this trypanosome, and probably not even a facultative host. It is interesting to note in this connection that a small Simulid fly (species so far unidentified) was found relatively frequently on the fowls in certain parts of Bugerere. On one occasion at Endeba, where the fly was more especially numerous, one specimen was found well infected with a trypaniform flagellate. The fowls in the enclosure were found to be infected with *T. gallinarum*. The flagellates in the Simulid were of small size and mostly crithidial in type, though a few showed the nuclear relations characteristic of trypanosomes.

"No connection between the fowl-parasite and that of the Simulid has been as yet established, but from the habits of the fly it is highly probable that it might play some part in the transmission of the trypanosome in question.—MURIEL ROBERTSON."

Are Ducks capable of Acting as a Reservoir of T. gambiense?

These experiments were undertaken on the same lines as those carried out by the 1908 to 1910 Commission with the Uganda fowl. It seems possible that ducks might react differently to *T. gambiense,* and throw some light on the part played by the enormous numbers of aquatic birds along the shores of Victoria Nyanza in relation to Sleeping Sickness.

Two Muscovite ducks were obtained from Mr. Walsh's farm, as wild birds were not procurable alive. The method of procedure followed consisted in first feeding laboratory-bred flies upon the ducks to ascertain whether the latter harboured any natural infection. The birds were then fed upon by infected flies and afterwards again tested with clean flies, and finally by blood inoculation into a susceptible animal. The blood was also examined from time to time microscopically.

Are the newly arrived Ducks capable of Infecting Laboratory-bred Flies with Flagellates.

Experiment 153.—From June 16 to 20, 1911, 45 clean laboratory-bred flies were fed upon the two ducks, two days on each bird. The flies were then fed for 27 days on a clean monkey and finally dissected. No flagellated flies were found.

Table III.

Expt. No.	Period for which flies fed upon Duck 144.	Number of flies.				Length of experiment in days.	Result.
		1st day.	30th day.	Dissected.	Containing flagellates.		
193	July 10—17, 1911	64*	54	64	0	50	—
236	Aug. 1—3, 1911 ...	81*	63	81	0	36	—
299	Aug. 16—19, 1911	89*	82	89	0	42	—
594	Jan. 1—16, 1912 ...	111*	53	111	0	32	—
	Totals	345	252	345	0		

* Fed upon clean monkey throughout the experiment.

Remarks.—All these experiments proved negative, no flagellates being found in the dissected flies.

From June 19 to 24, 1911, duck Experiment 144 was fed upon daily by flies known to be infective for *T. gambiense.* After a lapse of 16 days laboratory-bred flies were fed upon Duck 144, as shown in the preceding table.

On January 16, 1912, Duck 144 was killed and 6 c.c. of its citrated blood injected into a monkey. This monkey remained healthy and never showed trypanosomes in its blood.

Conclusions.

1. That *T. gallinarum* can multiply in the gut of *G. palpalis,* although this fly is probably not the normal host.

2. That Muscovite ducks are not capable of acting as a reservoir for *T. gambiense.*

DESCRIPTION OF PLATE.

The figures were all drawn at a multiplication of approximately 3000 diameters with the aid of the drawing apparatus of Abbé.

Fig. 1. Division stage showing the double appearance of the kinetonucleus and the two trophonuclei.

Fig. 2. Division stage showing centrodesmose of the trophonucleus spindle.

Fig. 3. Slender long form.

Fig. 4. Division stage showing centrodesmose of the kinetonuclear division apparatus; note double appearance of the kinetonuclei.

Fig. 5. Short, broad form from proventriculus.

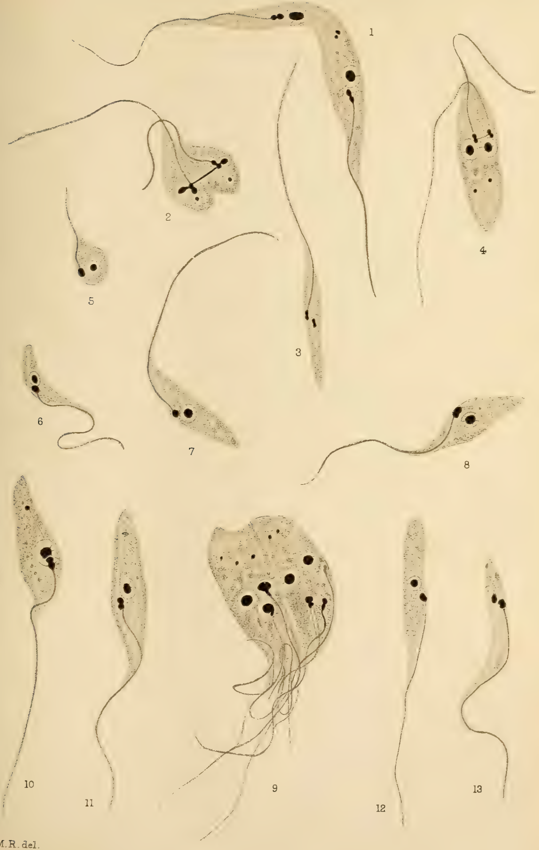
Figs. 6 and 7. Other forms from proventriculus.

Fig. 8. Typical broad form showing posterior granule and lines to trophonucleus and kinetonucleus.

Fig. 9. Multiple division.

Figs. 10 and 11. Very typical forms from the ordinary type of gut infection.

Figs. 12 and 13. Slender forms from proventriculus; these forms are also found further back in the mid and hind gut.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The Origin and Destiny of Cholesterol in the Animal Organism. Part IX.—*On the Cholesterol Content of the Tissues, other than Liver, of Rabbits under Various Diets and during Inanition.*

By G. W. Ellis and J. A. Gardner.

(Communicated by Dr. A. D. Waller, F.R.S. Received May 9,—Read June 20, 1912.)

(From the Physiological Laboratory of the University of London, South Kensington.)

In Part VIII of this series of papers, we published analyses of the livers of rabbits fed on various diets, and also of rabbits kept in a state of inanition. The results appeared to lend support to the hypothesis previously suggested with regard to herbivora, that when cells are broken down in the normal life process, their cholesterol is not excreted as a waste product, but is utilised in the formation of new cells. A function of the liver is to break down dead cells and eliminate their cholesterol in the bile. After the bile has been passed into the intestine in the process of digestion, the cholesterol is re-absorbed, possibly in the form of esters, along with the bile salts, and is carried in the blood stream to the various centres and tissues, for re-incorporation into the constitution of new cells.

In this paper we give the cholesterol contents of some of the other tissues of the animals dealt with in Part VIII, in which paper we gave a full account of the methods used in extracting the tissues, and for estimating the free and ester cholesterol. That paper also contained the detailed protocols, giving the weights of the animals during the various dietetic periods and during inanition, so that it is not necessary to refer to them in detail again. The tissues examined are blood, muscle, brain, kidney, and lung. The other organs of the rabbit were too small to be dealt with in individual animals.

The investigation comprises the following experiments, the data of which we have given as concisely as possible. Fuller details of the diets and conditions of the animals under experiment are given in our previous communication above mentioned.

The Cholesterol Content of Blood, Kidneys, Muscle, Lungs, and Brains of Rabbits Fed on Green Food.

Experiments I and II:—

Rabbit A was fed, for a period of about 30 days, on a diet of cabbage and very small quantities of extracted bran. It retained a healthy condition and constant weight.

The *blood*, of which 101·3 grm. were obtained, yielded as total cholesterol 0·2440 grm. of digitonin cholesteride, and as free cholesterol 0·2302 grm. of the compound. These figures represent a percentage of 0·0552 free cholesterol and 0·0033 ester cholesterol.

The *kidneys* were cut in a few pieces, rinsed with saline, and freed from adhering moisture by means of blotting paper. They weighed 20·72 grm., and yielded as total cholesterol 0·1390 grm. of digitonin cholesteride, and as free cholesterol 0·1080 grm. of the compound. These figures represent a percentage of 0·2534 free cholesterol and 0·0728 ester cholesterol.

Muscle. 100 grm. were taken for the estimation, the sample being cut away from the body wall and limb muscles. The total digitonin cholesteride was 0·1545 grm. and the free was 0·1360 grm., corresponding to a percentage of 0·0661 free and 0·0090 ester cholesterol.

The *lungs* were also washed with saline and freed from adhering moisture by means of blotting paper. Their weight was 9·11 grm. The total digitonin cholesteride was 0·0765 and the free was 0·0473 grm., corresponding to a percentage of 0·2524 free and 0·1558 ester cholesterol.

The *brain*, dissected out as completely as possible, weighed 10·592 grm. It was dried with sand and plaster of Paris in the usual manner. One-quarter of the ether extract was taken for the free cholesterol estimation, and half of the extract was saponified, of which one-half was taken. This was found advisable as it was more convenient to deal with small quantities of the compound. The total digitonin cholesteride was 0·2110 grm. and the free 0·2310. The free cholesterol was thus 2·12 per cent., and no ester was present.

Rabbit B received the same diet as A of the previous experiment. It was fed for a period of about seven weeks, and appeared in a normal healthy condition when killed. As the organs were treated in a precisely similar manner to those of the previous experiment, we shall merely give the figures obtained from the analysis.

The *blood;* 89·6 grm. were taken for the estimation. Half the ether extract yielded 0·1285 grm. of cholesteride, which corresponds to 0·0697 per cent. free cholesterol, and the saponified half yielded 0·1429 grm. of cholesterol, corresponding to a total of 0·0775 per cent. cholesterol and, by difference, 0·0078 ester cholesterol.

The *kidneys* weighed 15·65 grm. Half the extract yielded 0·0505 grm. of cholesteride, equal to 0·1568 per cent. free cholesterol. The saponified half yielded 0·0857 grm. of cholesteride, equal to 0·2661 per cent. total cholesterol and, by difference, 0·0093 per cent. ester cholesterol.

Muscle. 100 grm. taken. Half the extract yielded 0·0888 grm. of cholesteride, equal to 0·0635 per cent. free cholesterol. There was no ester cholesterol.

The *lungs* weighed 9·42 grm. Half the extract yielded 0·0962 grm. of cholesteride, equal to 0·4863 per cent. free cholesterol. The saponified half yielded 0·1084 grm., equal to 0·5593 per cent. total cholesterol, and, by difference, 0·0730 per cent. ester cholesterol.

The *brain* weighed 10·63 grm. One-fourth of the extract yielded 0·3070 of cholesteride, corresponding to 2·8796 per cent. free cholesterol. There was no ester.

Cholesterol Content of those Organs of Rabbits fed on Bran which had been Thoroughly Extracted with Ether to Remove all Fat and Phytosterols, and of Rabbits fed on the Same Diet with Added Cholesterol.

Experiment IV:—

A healthy doe rabbit fed on this diet for two weeks. Its weight remained constant.

The *blood*; 74·7 grm. were taken. The unsaponified half yielded 0·0620 grm. of cholesteride, corresponding to 0·0403 per cent. free cholesterol. There was no ester.

The *kidneys* weighed 12·675 grm. The unsaponified half yielded 0·0925 grm. of cholesteride, corresponding to 0·3547 per cent. free cholesterol. The saponified half yielded 0·0943 grm. cholesteride, corresponding to 0·3614 per cent. total cholesterol, and, by difference, 0·0067 per cent. ester cholesterol.

The *muscle*; 100 grm. taken. The unsaponified half yielded 0·0900 grm. of cholesteride, equal to 0·0437 per cent. free cholesterol. There was no ester.

The *lungs* weighed 8·31 grm. The unsaponified half yielded 0·0937 grm. of cholesteride, equal to 0·5481 per cent. free cholesterol. There was no ester.

The *brain* weighed 9·84 grm. One-fifth of the unsaponified half yielded 0·1638 grm. of cholesteride, equal to 2·0225 per cent. free cholesterol. There was no ester.

Experiment V:—

A healthy rabbit was fed on the extracted bran with cholesterol. During 19 days it received 1480 grm. of bran and 4·8 grm. of cholesterol. There was a loss in weight of 0·2 kgrm.

The *blood*; 91·2 grm. were taken. The unsaponified half of the extract yielded 0·1162 grm. of cholesteride, equal to 0·0619 per cent. free cholesterol. There was no ester cholesterol.

The *kidneys* weighed 14·645 grm. The unsaponified half yielded 0·0629 grm. of cholesteride, equal to 0·2296 per cent. free cholesterol. The saponified half yielded 0·0842 grm. of the cholesteride, equal to 0·2794 per cent. total cholesterol, and, by difference, 0·0498 per cent. ester cholesterol.

The *muscle;* 100 grm. were taken. The unsaponified half yielded 0·0928 grm. of cholesteride, equal to 0·0451 grm. of free cholesterol. The saponified half yielded 0·1075 grm. of the cholesteride, equal to 0·0522 per cent. of total cholesterol, and, by difference, 0·0071 per cent. of ester cholesterol.

The *lungs* weighed 12·25 grm. Half of the unsaponified half yielded 0·0446 grm. of cholesteride, equal to 0·3538 per cent. free cholesterol. Half of the saponified half yielded 0·0622 grm. of cholesteride, equal to 0·2488 per cent. total cholesterol. The ester cholesterol was, by difference, therefore, 0·0404 per cent.

The *brain* weighed 9·8 grm. One-half of the unsaponified half yielded 0·2892 grm., equal to 2·3368 per cent. free cholesterol. There was no ester cholesterol.

Experiments VII and VIII:—

In this case the cholesterol was injected in olive oil solution into the peritoneal cavity. 1 grm. was injected. A control experiment was performed injecting pure olive oil only. Both animals lost about 1 kgrm. in weight.

The *blood;* the control yielded 76·55 grm. Half the extract yielded 0·0963 grm. cholesteride, equal to 0·0611 per cent. free cholesterol. The saponified half yielded 0·0963 grm. cholesteride, equal to 0·0637 per cent. total. By difference the ester cholesterol is 0·0026 per cent. The cholesterol-injected animal yielded 81·895 grm. of blood. Half the saponified extract gave 0·1435 grm. cholesteride, equal to 0·0849 per cent. total cholesterol. The ester was not estimated through an accident.

The *kidneys;* the control kidneys weighed 14·53 grm. The unsaponified extract yielded 0·0565 grm. cholesteride, equal to 0·1890 per cent. free cholesterol. The saponified half yielded 0·0892 grm. cholesteride, equal to a total of 0·2985 per cent. cholesterol. The ester cholesterol was, therefore, 0·1095 per cent.

The kidneys from the cholesterol-injected animal weighed 15·98 grm. The unsaponified whole extract yielded 0·1050 grm. of cholesteride, equal to 0·1597 per cent. free cholesterol, and the filtrate after saponification 0·1227 per cent. cholesteride, equal to 0·1966 per cent. ester cholesterol. The total is, therefore, 0·3562 per cent.

Muscle; 150 grm. were taken from the control rabbit. The unsaponified extract yielded 0·1299 grm. of cholesteride, equal to 0·0421 per cent. free cholesterol. The saponified extract yielded 0·1820 grm. of cholesterol, equal to 0·0584 per cent. total cholesterol, and, by difference, the ester was 0·0163 per cent.

150 grm. were taken from the cholesterol-injected animal. The unsaponified half yielded 0·1844 grm. of cholesteride, equal to 0·0299 per cent. free cholesterol. The saponified half yielded 0·3868 grm. of cholesteride, equal to 0·1253 per cent. total cholesterol; the ester is, therefore, 0·0656 per cent.

The *lungs* were not examined in these cases.

The *brains;* from the control rabbit the brain weighed 11·927 grm. Half the saponified half yielded 0·3530 grm. of cholesteride, equal to 2·428 per cent. free cholesterol. There was no ester. The brain from the cholesterol-injected rabbit weighed 15·98 grm., 0·2277 grm. of cholesteride was obtained, equal to 2·2794 per cent. free cholesterol.

Cholesterol-content of these Organs of Rabbits during Inanition.

Experiment IX:—

A fat rabbit (I), of 3 kgrm. weight, fed for a few days on bran, then kept free of food for six days, water being allowed.

Experiment X:—

A thin rabbit (J), of 1·9 kgrm. weight, similarly treated.

The *bloods;* from Rabbit I were obtained 71·03 grm. of blood. The unsaponified half yielded 0·1523 grm. of cholesteride, equal to 0·1042 per cent. of free cholesterol. The saponified half yielded 0·1956 grm. of cholesteride, equal to 0·1338 per cent. total cholesterol; the ester was, therefore, by difference, 0·0296 per cent.

From Rabbit J were obtained only 40 grm. of blood. The unsaponified half yielded 0·1003 grm. of cholesteride, equal to 0·1219 per cent. free cholesterol. The saponified half yielded 0·1424 grm. of cholesteride, equal to 0·1730 per cent. total cholesterol. The ester cholesterol was therefore, by difference, 0·0512 per cent.

The *kidneys* from Rabbit I weighed 15·48 grm. The unsaponified half of the extract yielded 0·1295 grm. of cholesteride, equal to 0·4009 per cent. free cholesterol. The saponified half yielded 0·1867 grm. of cholesteride, equal to 0·4562 per cent. total cholesterol. The ester, by difference, was, therefore, 0·0698 per cent.

From Rabbit J the kidneys weighed 10·93 grm. The unsaponified half of the extract yielded 0·0869 grm. of cholesteride, equal to 0·3864 per cent. free cholesterol. The saponified half yielded 0·1026 grm. of cholesteride, equal to

0·4562 per cent. total cholesterol. The ester cholesterol was therefore, by difference, 0·0698 per cent.

The *muscles;* from I were taken 125·5 grm. The unsaponified half of the extract yielded 0·1585 grm. of cholesteride, equal to 0·0614 per cent. free cholesterol. The saponified half yielded 0·1793 grm. of cholesteride, equal to 0·0694 per cent. total. The ester cholesterol was, by difference, therefore, 0·0080 per cent.

From J 110·58 grm. were taken. The unsaponified half yielded 0·1680 grm. of cholesteride, equal to 0·0738 per cent. free cholesterol. The saponified half yielded 0·1690 grm., equal to 0·0743 per cent. total cholesterol. The ester cholesterol was, therefore, 0·0005 per cent.

The *lungs* from Rabbit I weighed 8·97 grm. The unsaponified half of the extract yielded 0·1536 grm. of the cholesteride, equal to 0·4246 per cent. free cholesterol. The saponified half yielded 0·1637 grm., equal to 0·4526 per cent. total cholesterol. The ester was, therefore, 0·0279 per cent.

From Rabbit J the lungs weighed 5·155 grm. The saponified half yielded 0·0669 grm. of cholesteride, equal to 0·6307 per cent. free cholesterol. There was a trace of ester cholesterol.

The *brain* from Rabbit I weighed 10·89 grm. One-fourth of the extract yielded 0·2682 grm. of the compound, corresponding to 2·3938 per cent. free cholesterol. There was no ester.

From J the brain weighed 10·59 grm. One-fourth of the extract yielded 0·2310 grm. of the compound, corresponding to 2·0617 per cent. free cholesterol. There was no ester cholesterol.

Bile.—Attempts were made to estimate the cholesterol content of the bile of the rabbits under different diets and during starvation. In the case of the normal animals, whether fed with green food or extracted bran, it was possible to detect cholesterol, but quite impossible to estimate gravimetrically with any approach to accuracy. The amount of bile in the gall bladders in each case was only 0·5—1 grm. in weight. In one or two cases the results were interesting. The contents of the gall bladder of the cholesterol and olive oil injected rabbit was found to be quite solid. Its weight was 1·72 grm. and contained 1·266 per cent. free cholesterol and no ester. The bile of the rabbit injected with olive oil only weighed about 3·66 grm. and contained 0·081 per cent. total cholesterol with a trace of ester cholesterol. In the former case the large excess of cholesterol in the bile was not wholly re-absorbed; an examination of the fæces from the animal showed that 7·3 mgrm. of cholesterol was excreted per day, as mentioned in our previous communication.

The biles from the two rabbits kept in a state of inanition contained a higher percentage of cholesterol than the normally fed rabbits. From Rabbit I were obtained 1·18 grm. of bile, yielding 0·5457 per cent. free and 0·3151 per cent. ester cholesterol. From Rabbit J the bile weighed 1·39 grm., yielding 0·1678 grm. free and 0·1329 ester cholesterol.

Discussion of Results.

Blood.—In Table I we gather together the results of estimations of cholesterol and cholesterol esters in the blood of rabbits under the various diets given in this and in a former paper. The results show that the cholesterol-content of the blood after food depends on the sterol-content of the food taken. In the case of animals in a state of inanition, which are living on their own tissues, the blood, just as in the case of the liver, contains an increased amount of both free and compound cholesterol.

The results throw no light on what governs the relations of free and combined cholesterol.

Muscle.—The cholesterol-content appears to bear no relation to the sterol-content of the diet. In the cabbage-fed and in the starving animals, the mean total cholesterol in each case is 0·0693 and 0·0718. The total cholesterol appears to vary from 0·0437 to 0·0751 per cent. The results throw no light on the variations of the relative proportions of free and combined cholesterol.

Brain.—The percentage of free cholesterol varied in different animals from 2·02 to 2·88. The mean of eight experiments was 2·315 per cent. In no case was any evidence of the presence of cholesterol-esters found.

Kidney.—The results do not indicate that the content of this tissue depends in any way on diet. The average values for the animals fed on cabbage and extracted bran, which were apparently in good health, are free cholesterol 0·2466 per cent. and ester cholesterol 0·0596, figures which are not very dissimilar from the mean of those given by Windaus* for normal human kidneys, viz., free 0·24, and ester 0·02 per cent. In the case of the starved animals the total cholesterol is much higher. The average figures in the two experiments are total cholesterol 0·5211 per cent., free 0·3937 per cent., and ester 0·1274. The increase above normal is very marked in the case of the ester.

In the case of the two rabbits which had had oil injected into the peritoneal cavity, and which were not in normal health, a similar increase in the ester cholesterol, compared with the free, is noticeable, the average

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* "Über die quantitative Bestimmung der cholesterin und der cholesterin colic in einigen normal und pathologischen Nieren," A. Windaus, 'Zeit. für physiol. Chem.,' 1910, vol. 65, p. 114.

Table I.—Percentage of Cholesterol Content of the Blood under Various Diets.

Diet.	Total free and combined cholesterol per 100 grm. of blood.	Free cholesterol per 100 grm. of blood.	Ester cholesterol per 100 grm. of blood.
Ordinary bran*	0·0768	0·0649	0·0119
,, ,, *	0·0877	0·0741	0·0136
Mean	0·0823	0·0695	0·0128
Green food, cabbage leaves, A	0·0585	0·0552	0·0033
,, cabbage stalks, B	0·0775	0·0699	0·0078
Mean	0·0680	0·0625	0·0056
Bran extracted by ether*	0·0675	0·0454	0·0221
,, ,, *	0·0677	—	—
,, ,, *	0·0553	0·0453	0·0100
,, ,,	0·0403	0·0403	nil
Mean	0·0577	0·0437	0·0107
Extracted bran and cholesterol*	0·0877	0·0492	0·0386
,, ,, ,, *	0·0895	0·0575	0·0320
,, ,, ,, *	0·0843	0·0653	0·0190
,, ,, ,,	0·0619	0·0619	nil
Mean	0·0809	0·0585	0·0224
Extracted bran (olive oil injected into peritoneal cavity)	0·0637	0·0611	0·0026
Extracted bran (olive oil and cholesterol injected into peritoneal cavity)	0·0849	lost	lost
Without food but allowed water (1)	0·1338	0·1042	0·0296
,, ,, ,, (2)	0·1731	0·1219	0·0512
Mean	0·1535	0·1131	0·0404

* Part VII, 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 565.

values being—free cholesterol 0·1744 per cent., and ester cholesterol 0·153 per cent. These high ester values recall the high ester value found in human pathological kidneys by Windaus.

Lung.—The cholesterol-content, as one would expect, has nothing to do with the diet. It is interesting to note, however, that this tissue has a higher cholesterol-content than any we have examined, with the exception of brain. In six animals the average total free and combined cholesterol is 0·5022 per cent., the free cholesterol 0·4493 per cent., and ester cholesterol 0·0529. The ester cholesterol was very variable and, in two cases, nil.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

A Note on the Protozoa from Sick Soils, with some Account of the Life-Cycle of a Flagellate Monad.

By C. H. Martin, M.A., late Demonstrator in Zoology at Glasgow University and at the Imperial College of Science.

(Communicated by Prof. E. A. Minchin, F.R.S. Received May 10,—
Read June 20, 1912.)

[Plate 10.]

Of recent years a great deal of attention has been attracted to the subject of sick soils, *i.e.* soils of which the produce has fallen off without the decrease being definitely attributable to the lack of plant-food in the soil. In this preliminary note I should like to deal shortly with some aspects of this question from a zoological point of view, and also to describe the life-cycle of a flagellate monad which happened to be one of the organisms from this source, of which I have succeeded in obtaining pure animal cultures. I hope to describe cultures of this and other forms in greater detail in a forthcoming paper.

The literature on this subject from a botanical and chemical standpoint will be found in a paper by Dr. Russel and Dr. Hutchison "On the Effect of Partial Sterilisation of Soil on the Production of Plant-Food,"* and it is particularly to this work that we owe the insistence on a hypothesis which is full of interest to the zoologist, namely, that the activity of protozoa in these soils may in many cases be a factor in bringing about soil-sickness.

It is obvious that one of the first steps in the discussion of this problem must be the elucidation of the normal protozoan population of a soil.

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* 'Journal of Agricultural Science,' 1909, vol. 3, Part II.

It must, I think, be clear that on the surface of ordinary soils practically any form of protozoan may be found either in the encysted or in the active condition. If small surface pools are formed on well-manured soil it is obvious that a large number of protozoa, including the larger ciliates, may thrive. The question of the ciliates has already been dealt with by Mr. Goodey,* who was, as far as I am aware, the first to describe some of the protozoa of sick soil. But it seemed to me that, in spite of his paper, much remained to be done, particularly in connection with the smaller amœbæ and the flagellates, which must, I believe, play the most important *rôle* in the causation of this phenomenon. It seems probable *à priori* that the most common limiting factor as regards the activity of protozoa in the soil is the average quantity of water. It is generally assumed that in a fairly dry soil—that is, a soil that feels quite dry to the touch—there is 20 per cent. of water by volume; but as this water is largely present in the soil as a surface-film on the small soil particles, it is evident that the protozoa capable of leading a trophic life under these conditions must be either of a very small size or else must have developed some particular protective mechanism against desiccation. It seems to me that there are two possibilities for a protozoon that is going to do any marked harm in a soil during a year (on the assumption that Russel and Hutchison's hypothesis that soil-sickness is due to the ingestion of soil-bacteria by protozoa)—either the protozoon must be satisfied with a very low percentage of water in the soil during its trophic life, or else it must have a capacity of readily encysting, together with the capacity of reproducing with enormous rapidity as soon as the soil becomes saturated with the necessary amount of moisture. It is quite possible that all the harmful protozoa will be found to possess both the above faculties to a certain degree—probably coupled, if they are to make the best use of the water of the surface-film, with a small size. In the case, however, of the soils of sewage farms, which are frequently saturated with water, any of the protozoa, even the larger ciliates, would be capable of acting with a certain amount of efficiency as a check upon bacterial life. In order to limit the problem, I decided to examine a soil which, though probably never very dry, was yet never in the waterlogged condition of the soil on a sewage farm. Dr. Russel was kind enough to send me samples of sick soils from three different sources, and of these I have devoted most of my attention to the sample derived from a sick tomato bed.

For the purpose of separation of the organisms I used various types of agar-plate, and as much still remains to be done from the cultural point of view, I have decided to publish the full details of my methods in the later paper. It must, however, be evident that the attempt to solve the problem of how far any protozoon is really doing damage under the normal soil conditions from a study of the forms grown on agar-plates must be a very difficult and dangerous one. It is more than probable that the sample of population cultivated under all the present methods may not by any means be a fair representation of the population of the given soil. On the other hand, it is, I think, important to observe that one finds that each small sample of a given soil for each culture-condition has given rise to a fairly constant specific fauna, whereas samples on other soils under these conditions have also given rise to constant but quite distinct faunas. If these facts are taken into consideration, it seems hard to believe that all these forms are a purely negligible factor in the question of soil-sickness, since it is important to note that many of these forms are new and—a point of difference from the ciliates—have never previously been found in fresh-water pools or infusions. It is, therefore, unlikely that all these organisms are present in the soil as cysts, because they must presumably lead a trophic life somewhere, and it seems probable that their trophic activity is a cause of soil-sickness. On the other hand, it is quite possible that the form or forms which are most dangerous in cases of soil-sickness may not up to the present have survived under the conditions of plate-cultures. I do not think that this view is probable, but the question can, of course, only be decided by parallel cultures of three kinds: (1) Plants grown on the sterilised soil; (2) plants grown on the sterilised soil to which pure cultures of the various protozoa, with the bacteria on which they feed, have been added; and (3) plants grown on the sterilised soil to which cultures of the protozoa without the bacteria have been added.

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* 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 165.

As regards methods of preparation, wet cover-slip smears were solely used. In this connection a device suggested to me by Mr. Jamieson, of the Natural History Department, Glasgow University, has proved extremely useful. It is obvious that with a large number of cultures on one's hands a number of smears must be fixed which cannot be immediately stained. The smears in these cases after fixation were stacked in tubes in 70-per-cent. alchol. It was found, however, in some cases, after the smears had been some time in the tubes, that in the case of thin smears it was extraordinarily hard to tell the right from the wrong side of the cover-slip. I therefore got some oblong cover-slips of which one corner was cut off, made by Messrs. Frazer, of Edinburgh. It will, I think, be obvious that, if one makes up one's mind to always have the smear on the lower surface of the cover-slip when the top right-hand corner is cut, no mistake of this kind can possibly arise.

I should like to take this opportunity of thanking Dr. Russel for his kindness in sending me samples of soil, and for much advice, and also

Prof. Minchin for allowing me to write up these notes in his laboratory at the Lister Institute.

Of the forms which I cultivated from the sick tomato-soil a rough list will be found at the end of the paper. In this note I wish to describe a flagellate which passes through its life-cycle in my cultures, terminating in the formation of a zygote-cyst,* with great regularity. I do not propose to enter into the question of the nomenclature of this form in any detail here. It appears to me to be identical with the flagellate figured by Stein as *Cercomonas termo*, which he identifies with *Monas termo* of Ehrenberg, and which is therefore presumably identical with the *Oikomonas termo* of Bütschli. The trophic form of this organism is a roughly spherical animal, provided at its physiologically anterior end with a single, rather short, thick flagellum (Plate 10, fig. 1).

The size of the animal may be somewhat variable. In young cultures, in which no gametes occur, the active forms seem to measure about $4{\cdot}5\,\mu$, whereas in the older cultures larger forms are met with. The body, particularly at its posterior end, contains large rounded vacuoles. These vacuoles may disappear, and are probably mostly food-vacuoles, and I cannot find any evidence for the existence of a true contractile vacuole. In life, the posterior end of the body may undergo amœboid movement, and, more particularly in actively swimming forms, the body may assume a rather more elongated shape than in the resting form (fig. 2). The body is often found crowded with ingested bacteria, and a number of small granules which appear to stain reddish in neutral red. In life, a nucleus, with a fairly large caryosome, can be distinguished clearly. In stained forms the flagellum can be seen to arise from quite a large blepharoplast, which stains vividly in iron-hæmatoxylin. The nucleus in the stained preparations is large and vesicular. Most of the chromatin appears to be contained in the caryosome, but a certain amount seems to be present on the nuclear wall (fig. 1).

The division in this monad seems to be initiated by the division of the blepharoplast (fig. 3), but this is quickly followed, or possibly in some cases preceded, by the splitting of the flagella. Up to the present I have found no evidence for the outgtowth of new flagella in the dividing forms of this animal. The nucleus up to this stage presents its characteristic appearance of a dark caryosome lying in a large nuclear space. It quickly becomes much darker, and the whole space is crowded with relatively large chromatin granules (fig. 4). A curious feature in this division is the apparent increase in the amount of chromatin in the nucleus during the early stages of division, an increase which it seems difficult to account for when one considers the amount of chromatin in the caryosome and on the wall of the resting nucleus (fig. 5). One of the division-products of the blepharoplast, with its flagellum, now passes around the nucleus, being still connected with the other blepharoplast by a faintly staining strand, which seems to have more affinity for eosin than for chromatin stains (*vide* figs. 5 and 6).

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* It is unfortunately impossible at present to prove conclusively that this process is not an autogamy, since up to the present I have not carried on continuous observations on conjugating forms in life.

The chromatin granules are now massed in an irregular oval body on the strand connecting the two blepharoplasts, as is shown in figs. 5 and 6. The chromatin masses then appear to stream in an irregular manner towards either blepharoplast. There they become collected into two crescent-shaped masses, in the convexity of each of which a blepharoplast lies (fig. 7). At the same time the body of the flagellate becomes much elongated in the axis of the line joining the two blepharoplasts. About this stage there seems to be mysterious diminution in the amount of chromatin comparable to the increase which was noted in the early stages of division. The body of the flagellate now becomes constricted (fig. 8) and the division-products separate (fig. 9). It seems probable, from the relative frequency with which the stages are met on the stained films, that the early stages of division are much slower than the later.

Before proceeding to a description of the process of encystation in this flagellate, I should like to draw attention to some features in the behaviour of the cultures with regard to the life-cycle of this animal. In the first place, the appearance of an encystation-epidemic in any particular culture seems to be primarily due to the amount of moisture on the culture-plate. I have, on several occasions, started series of three parallel cultures: (*a*) rather dry, (*b*) fairly moist, and (*c*) quite moist. In all three the organisms would do well at a temperature of about 18° C., with the result that at the end of the third day the fixed films of any culture would show a large number of division-figures. An encystation-epidemic would, however, set in in (*a*) at the end of a week, in (*b*) at the end of about 12 days, whereas cultures of the type of (*c*) have been kept over two months without the appearance of encystation.

What the ultimate factor in this appearance of encystation-epidemics in drying cultures may be I should not at present care to hazard an opinion. Possibly it may be simply a direct osmotic effect upon the animal, possibly it may introduce changes in the bacterial flora of the plate resulting in a diminution of the appropriate food-supply of the animal, or possibly it may initiate the formation of some substance by the bacteria toxic to the flagellate and to which it reacts by encystation. But it is evidently useless attempting to analyse the physiology of this phenomenon without a great deal more experimental evidence than I have at present collected.

I will only draw attention to one other feature in the physiology of these cultures before proceeding with my account of the morphological features presented in the formation of the zygote-cyst. On several occasions I have attempted to make sub-cultures of this flagellate from flourishing cultures in which few or no cysts were present. These cultures always failed. Whether this failure was primarily due to a zoological or a bacteriological factor I am at present unable to say, but sub-cultures made from the same primary cultures at a later stage when the cysts had been formed were uniformly successful.

The conjugation in this flagellate is probably isogamous, and there seems to be a certain amount of evidence tending to show that the gametes are characterised by a slightly larger size of body and by a slightly smaller nucleus containing a relatively smaller caryosome. The gametes usually contain quantities of ingested bacteria, and it would seem that this pronounced trophic activity constantly precedes conjugation. The gametes seem to come into contact laterally, and the zygote formed by their fusion swims around actively for some time by means of its two flagella (figs. 10 and 11), which seem to be always inserted near the same pole. The zygote now grows in size, the growth appearing to be largely due to the formation of a large vacuole in the posterior portion of the body. At a slightly later stage this vacuole is found to be occupied by a solid round body, which I am inclined to regard as a reserve-body (figs. 13, 14). The flagella now disappear, and I have not yet succeeded in tracing the fate of the blepharoplasts in the later stages of the zygote.

The zygote now secretes a wall, and presents a very characteristic appearance with its two nuclei closely applied to the anterior wall of the large vacuole containing the reserve-body. The reserve-body is best shown in early cysts stained with iron-hæmatoxylin, which stains it a faint yellowish colour. The ripe cysts can only be stained with great difficulty with acid hæmatoxylin. In these permanent preparations the reserve-body cannot be seen (figs. 15, 16). The nuclei apparently now undergo some process of reduction division, as is shown in figs. 13 and 14. But I hope to return to this point in my final paper. The ripe zygote-cyst (*vide* fig. 16) contains a single nucleus, the result of the fusion of the nuclei of the two gametes. To the question of some of the peculiar features displayed by this life-cycle, and the light which they may throw on the affinities of this flagellate, I hope to return in my forthcoming paper.

In addition to these monads I have succeeded in obtaining pure animal cultures of a number of other forms. Amongst these some have been previously described by other workers, and I give a short list of the forms that I have up to the present identified :—

(1) *Chlamydophrys stercorea;* (2) *Amœba diploidea;* (3) *Amœba sp.?* (4) *Amœba sp.?* (5) *Amœba sp.?* (6) *Copromonas;* (7) *Bodo sp.?* (8) *Prowazekia sp.?* (9) *Astasia sp.?*

To some of these forms I hope to return in my later paper, but I should like to point out that I have now worked for over a year with cultures of a form which seems to me absolutely identical with *Chlamydophrys stercorea*, and have entirely failed to find any indication in confirmation of Schaudinn's account of the sexual stages of this form. This is the more difficult to understand as the account and the figures which he has published of the asexual stages are in complete accord with those I find in my cultures. Schaudinn, as will be remembered, stated* that in all his cultures, at the end of a few days, a formation of flagellate gametes set in, which after an isogamous conjugation gave rise to small zygote-cysts. These cysts, according to his account, could only be further cultured after the passage through the gut of some vertebrate. My cultures, under a large number of different conditions, have uniformly ended in a cyst formation without any sexual process, and these cysts could always be readily cultured on all fresh media, and would not pass in a living condition through the gut of a fowl. I have found flagellates in some of my early *Chlamydophrys*-cultures, but I hope to be able to show in my forthcoming paper that these are parasites, and have no connection with the life-cycle of *Chlamydophrys.*

EXPLANATION OF PLATE.

All the figures were drawn with a camera lucida under Zeiss comp. oc. 18 and 1·5 apochr. objective.

All the figures except 1, 2, 11, and 12 were drawn at table level.

1. Normal round form of *Monas termo.*
2. Active elongate form.
3. Early stage of division ; the flagella and blepharoplasts have divided.
4. Slightly later stage of division ; the blepharoplasts with their attached flagella are beginning to separate, and the nucleus is filled with a number of spherical masses of chromatin.
5. The blepharoplasts with their attached flagella have moved apart so that the enlarged nucleus filled with the chromatin-spheres lies between them. The blepharoplasts are still united by a thread which stains faintly with eosin.

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* "Untersuchungen über die Fortpflanzung einiger Rhizopoden," 'Arb. a. d. kais. Gesundheitsamt,' 1903, vol. 19, p. 547.

6. Later stage of division; the chromatin-spheres are massed on the thread joining the blepharoplasts.
7. The chromatin-masses have passed to either pole of the dividing monad; the line joining the blepharoplasts still persists.
8. Division is almost complete, and the line between the blepharoplasts has disappeared.
9. One of the products of a recent division; the nucleus has not yet been reconstructed.
10. Early stage of conjugation.
11. Active zygote-stage showing the approximation of the blepharoplasts and large vacuoles.
12. Early stage of encystation of the zygote; the wall at this stage is quite soft and stains with eosin.
13. Unripe cyst, showing reserve-body. Iron-hæmatoxylin preparation.
14. Unripe cyst, showing possibly reduction-division on the part of the nuclei.
15. Almost ripe zygote cyst, with elongated synkaryon. Hæmalum-preparation; does not show reserve-body.
16. Ripe cyst, with round synkaryon. Hæmalum-preparation; does not show reserve-body.

Further Observations on the Variability of Streptococci in Relation to Certain Fermentation Tests, together with some Considerations Bearing on its Possible Meaning.

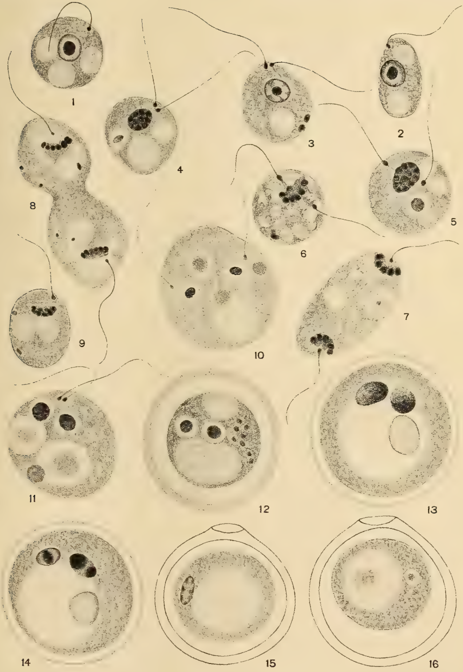
By E. W. Ainley Walker, M.A., D.M., Fellow and Tutor of University College, and Lecturer in Pathology in the University of Oxford.

(Communicated by Prof. Gotch, F.R.S. Received May 16,—Read June 20, 1912.)

(From the Department of Pathology, University of Oxford.)

The fermentation tests devised by Mr. H. Gordon (1905, 1) were employed by him and by a number of subsequent observers as a means of differentiating streptococci into definite varieties.

This application of the tests necessarily presupposes the stability of the reactions yielded by any given strain of streptococci in the test media. But after a prolonged examination of a number of different strains of streptococci I was unable to confirm this primary requirement. Consequently, I ventured to call in question the supposed constancy of the tests (1910, 2; 1911, 3). A considerable mass of evidence of the variability of streptococci under cultivation in respect of their reactions in "Gordon's media" was submitted. The observations recorded made it clear that the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E. Wilson, Cambridge.

reactions in these media of a series of selected strains of streptococci varied independently from time to time under the conditions of ordinary laboratory cultivation, and could in certain cases be made to vary very greatly by growing the organisms for longer or shorter periods in media containing particular "sugars."

The conclusions which were drawn from these and similar observations have been strongly controverted by Gordon (1911, 4), who issued an article containing a series of criticisms of my results, and reasserted his claim to have differentiated distinct types of streptococci by means of his tests. The grounds on which he based this contention, unfortunately, appear to involve some slight misapprehension of the question under discussion. This was whether the "types" thus differentiated are fixed and independent, or merely temporary and liable to variation and interchange of characters.

On the other hand, the later work of Beattie and Yates (1911, 5) completely confirmed the views which I had put forward, since they found it impossible to differentiate between the various strains of streptococci which they used, and state that in their hands "Gordon's tests have proved quite unreliable for this purpose."

For the purpose of throwing further light upon the subject, I have examined the behaviour of a single streptococcus freshly isolated from the human subject in regard to Gordon's tests, both after growth in certain ordinary culture media, and after a series of successive passages through the mouse. I have also incidentally re-examined the reactions of some of the streptococci employed in my previous experiments after the lapse of a further period of 18 months. The results obtained are in entire agreement with my former conclusions.

Methods.

The methods and precautions used were the same as those employed in my previous work (1911, 3). But, in view of certain doubts expressed by Gordon, it may be well to state that throughout the whole of my investigation of the fermentation tests the "sugars" used in the preparation of the media have been pure substances obtained from Merck. Further, in every case where the reaction appeared to give a negative result, the streptococci in the tube in question were proved by successful sub-culture to be actually living before the result was accepted as negative.

The streptococcus A was isolated *post mortem* from the blood of a case of ulcerative endocarditis, and appeared to be present in pure culture. It was three times plated out, and propagated on each occasion from a single isolated colony. Its reactions were then tested in Gordon's media.

A stock culture was established in stab agar, and propagated in sub-culture every week. Cultures were also established in bouillon, gelatin, and milk. These were all incubated at 37° C., and were sub-cultivated every three days—the bouillon culture into fresh bouillon, the gelatin culture into gelatin, and the milk culture to milk.

After the lapse of a month, and again after two months, the streptococci growing in bouillon, gelatin, and milk respectively were examined as regards their reactions in the test media. In the meantime the organism from the original stock was being passed through a succession of mice. It was recovered in each case *post mortem* from the blood of the mouse, plated for purity, tested in Gordon's media, and again inoculated into a fresh mouse. In this manner five successive passages were carried out, and the reactions of the streptococcus recorded after each passage.

Results.

In Table I are shown the reactions of the streptococcus A as originally isolated, and after its continued cultivation in bouillon, gelatin, and milk at 37° C. for periods of one month and two months respectively. It is seen that in every case the original reactions underwent important changes. Fifteen changes of individual reactions occurred in total, and four new sets of reactions made their appearance in the course of the experiment in question. The power of clotting milk was lost in every case, and only the neutral red, saccharose, and lactose reactions remained entirely unchanged. Thus, so far as the fermentation tests afford information, streptococcus A and its derivatives in bouillon, gelatin, and milk present five distinct varieties or strains of streptococcus.

Table I.—Showing the Reactions of Streptococcus A when first Isolated and after Cultivation in different Media.

Streptococcus A.	Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes after one month.	Changes after the second month.
	Acid.	Clot.									
When originally isolated………	+	+	0	+	+	+	+	0	0		
After 1 month in bouillon ……	+	0	0	+	+	+	+	+	0	2	
„ 2 months „ ……	+	0	0	+	+	0	0	+	+		3
After 1 month in gelatine ……	+	0	0	+	+	+	0	+	0	2	
„ 2 months „ ……	+	0	0	+	+	+	+	+	0		1
After 1 month in milk…………	0	0	0	+	+	+	+	0	+	3	
„ 2 months „ …………	+	0	0	+	+	0	0	+	+		4

In all the tables the sign + indicates the production of an acid reaction in the sugar medium, or the reduction of neutral red, or the production of a clot in milk where the property of clotting milk is under examination. The sign 0 indicates that the change in question did not occur within the limits of time prescribed for Gordon's tests.

Table II contains the results obtained by testing the streptococcus A in its original form, and after each successive passage through the mouse. There are eight changes of individual reactions. All six series of reactions are different, and each of them is different from the reactions shown in Table I from the cultures grown in bouillon, gelatin, and milk.

Table II.—Showing the Reactions of Streptococcus A when first Isolated and after each of Five Passages through the Mouse.

Streptococcus A.	Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Number of changes.
	Acid.	Clot.								
When originally isolated	+	+	0	+	+	+	+	0	0	
After one passage	+	+	0	+	+	+	+	+	0	1
„ two passages	+	0	0	+	+	0	+	0	0	3
„ three „	+	+	0	+	+	0	+	+	0	2
„ four „	+	+	0	+	+	+	+	+	0	1
„ five „	+	+	0	+	+	+	0	+	0	1

Thus it appears that, starting from a single freshly isolated streptococcus of undoubted purity, no less than ten distinct varieties, or variants, were obtained. The only instances of identical reactions which occurred were those exhibited by the variants obtained after two months' growth in bouillon and two months in milk, and the variants got by one month's growth in bouillon and two months' growth in gelatin.

In Table III are given the reactions of the streptococci L, P, G, M, V, E, in January, 1912, along with the results which they had yielded previously from stock agar cultures in 1908 and 1910, as shown in my previous papers. G is the only streptococcus which, in 1912, exhibits the same series of reactions as in 1910. Numerous changes are seen in the reactions of the streptococci. Streptococci S and H of my previous list could not be re-examined as they had unfortunately been allowed to die out. Streptococcus E was one originally isolated from horse-dung, all the others were of human derivation.

Table III.—Showing the Reactions of certain Streptococci at Different Dates after continued Cultivation in Stab Agar.

Streptococcus.	Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Number of changes.
L	June, 1908	+	+	0	0	0	0	0	
	„ 1910	0	0	+	0	0	+	0	4
	January, 1912	+	0	+	+	0	+	0	2
P	June, 1908	+	0	+	+	0	+	0	
	„ 1910	0	0	+	0	0	+	0	2
	January, 1912	+	0	+	+	0	+	0	2
G	June, 1908	+	+	+	0	+	+	+	
	„ 1910	+	+	+	+	0	+	0	3
	January, 1912	+	+	+	+	0	+	0	0
M	June, 1908	+	+	+	+	0	+	0	
	„ 1910	0	+	+	0	0	+	0	2
	January, 1912	+	0	+	+	0	+	0	3
V	June, 1908	+	+	0	0	+	+	0	
	„ 1910	0	0	+	0	0	+	0	4
	January, 1912	+	+	+	+	0	+	0	3
E	August, 1910	0	+	+	0	+	+	+	
	November, 1910	0	+	+	+	0	+	+	2
	January, 1912	+	+	+	+	+	+	+	2

Stability of the Reactions.

The evidence for and against the stability of Gordon's test reactions may now briefly be summarised.

In bringing out the tests originally Gordon stated (1905, 1) that he had "found on repeating the tests three times over that each of the ten streptococci responded on each subsequent occasion to each test in the same fashion as it had done in the first instance." He also wrote as follows (1905, 6): "On several occasions I have re-examined a streptococcus in the differential tests after it had been in culture *for periods up to a fortnight*,* and up to the present time I have always found that such streptococci respond to the test in exactly the same manner as when first isolated." He also re-examined 11 different streptococci after one passage through the mouse, and found variation in two cases only, affecting one reaction in each case. These results he regarded as "reassuring" in relation to the question of the stability of the reactions. Thus it is seen that the original evidence in favour of the stability of the reactions was somewhat slight.

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* The italics are mine.—E. W. A. W.

Andrewes and Horder (1906, 7), recognising that constancy of the tests was "clearly a cardinal point," investigated the question with considerable care. They eventually concluded that the reactions were sufficiently stable for the purpose which they had in view, though they frankly stated the existence of some degree of variation within their experience. Indeed, the evidence which they adduce of constancy of the reactions after prolonged sub-culture, after passage, and under varying external conditions, is in each case associated with an admission of the occurrence of instances in which the reactions exhibited variation.

Cumpston (1907, 8), who examined streptococci from the throat and tissues in scarlatina, 50 per cent. or more of which gave identical reactions, stated that he re-examined many of the cultures after periods ranging up to as much as three months, and was able to corroborate the assertion that each strain of streptococcus preserves its characters unchanged.

On the other hand, Beattie and Yates (1911, 5), who examined the same question, found one change of reaction in each of three strains of streptococci after prolonged sub-culture, and one or more changes of reaction in four strains of streptococci out of five after a single passage through the rabbit, one strain remaining unchanged.

In my own earlier experiments (1911, 3) streptococcus L exhibited seven different series of reactions in the course of the investigation, P six different series, S nine, G seven, M four, V three, and H two, while in the present observations streptococcus A has been found to offer no less than 10 distinct and independent series of reactions. The January, 1912, examination of the old streptococci adds a new series of reactions in the case of L, P, M, and V.

Thus it is clear that it may justifiably be stated (as I stated in my former paper) that streptococci, which are at one time different in respect of the tests, may at another time give identical reactions, while those which are apparently identical at a given date may later on exhibit totally different reactions. That is to say, the test reactions are by no means stable.

Gordon maintains emphatically (1911, 4) that variation on sub-culture does not discount the value of "approved types" of streptococci as defined by him. This attitude is extremely difficult to comprehend, since the question of stability of the reactions can only be approached at all by the examination of successive sub-cultures.

It will, of course, be admitted as a self-evident proposition that, if a given micro-organism varies in such a manner as to become entirely indistinguishable from another given micro-organism, the two (so long as they remain indistinguishable) can no longer be regarded as distinct varieties. Until some differentiating character has been discovered, they must be looked upon as identical. It has been shown in my experiments that these considerations apply to streptococci in respect of their reactions in Gordon's media. And it has also been shown that, *on sub-culture through the animal body*, as well as on sub-culture in artificial media, the test reactions of particular streptococci tend to exhibit striking variations.

The conclusion seems to follow unavoidably that Gordon's tests do not afford an adequate basis for a classification of streptococci. This conclusion, however, does not necessarily imply that the tests are valueless. Nor does it suggest that fixed and definite distinct varieties do not exist among the streptococci. But it maintains that the existence of such varieties has not been proved by the application of the tests, nor even rendered in the least degree probable.

Even had the tests been found to be reasonably constant under ordinary conditions, the results obtained by their use do not on close examination appear to supply so clear and decisive a differentiation of streptococci as might be supposed from writings on this subject. Thus, among six variants of the *S. equinus* described by Andrewes and Horder (1906, 7)—a type of streptococcus "which seems entitled to rank as a species" in their opinion—no less than three are identical with variants of their *S. pyogenes*, so far as Gordon's tests themselves afford any indication. The distinction which they actually draw between the organisms is made to rest on characters which form no part of Gordon's tests, such as pathogenicity, the presence or absence of growth in gelatin, and so forth.

Again, if one looks into the reactions actually laid down by Gordon himself in his latest communication (1911, 4) as typical of *S. salivarius*, *S. pyogenes*, and *S. fæcalis*, one finds a range of admitted possible variation such as brings *S. pyogenes* very near *S. salivarius* on the one hand, and equally near *S. fæcalis* on the other. For it is to be noted that, in the form in which the reactions are now given, we have two alternatives for *S. salivarius*, and no less than eight alternatives for *S. pyogenes* (see Table IV). Further, the only absolutely fixed distinction, as regards the test reactions between what may pass as a *S. pyogenes* and what is to be classed as a raffinose-negative *S. salivarius*, is the difference in the reaction to neutral red. Similarly, the only distinction in the test reaction between another form of *S. pyogenes* and the *S. fæcalis* is again the reaction in the neutral red test—a test which Andrewes and Horder have noted (along with salicin) as "more liable to vary than most of the others."

It appears, therefore, that even in these selected cases the types differentiated by the tests lack something in definition and distinction.

Table IV.—Showing the Reactions indicated by Gordon for certain "Approved Types" of Streptococci expressed in a Table.

Streptococcal type.	Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Number of possible forms allowed by this series of reactions.
	Acid.	Clot.								
Salivarius	+	+	+	+	+	+ 0	0	0	0	2
Pyogenes.........	+	0 +	0	+	+	0	0	+ 0	0 +	8
Fæcalis	+	+	+	+	+	0	0	+	+	1

Possible Meaning of the Reactions.

Hitherto, attention has been directed to the lack of constancy of the tests, and with that object examples have chiefly been used in illustration which showed a noteworthy degree of variability. But it is not to be supposed on that account that every streptococcus will at any moment necessarily exhibit variation. Within the limits of my own experiments, it has not always been possible to produce variation in particular streptococci even after several months of manipulation, though at another period, or under different conditions, the same streptococci frequently exhibited considerable variability. Again, streptococci which have given a series of variations over a considerable period may later on preserve an appearance of constancy even after the lapse of a long interval of time, as in the case of streptococcus G in agar culture in June, 1910, and January, 1912. But no streptococcus yet examined in these experiments has preserved unchanged the whole of its reactions throughout the whole period of observation.

We do not at present sufficiently understand the conditions governing streptococcal variation or the conditions of temporary constancy, but much of the evidence seems to point in the direction of a distinct environmental influence.

The question is not an easy one to investigate, owing to the difficulty of supplying absolutely constant conditions of environment even in the laboratory cultivation of streptococci. But it is at least possible that particular nutritive and other conditions in the environment favour the development of particular series of reactions. When a given streptococcus has reached the particular (or one of the particular) series of reactions favoured by particular surroundings, it is quite possible that under continued cultivation in the same surroundings it may exhibit no changes of reaction during long periods of time. And it might even be somewhat slow to change when introduced into a new environment.

It appears to me that this hypothesis of adaptation to the particular environment affords a more probable explanation of the differences observed among streptococci in the test reactions than the theory of fixed types. For example, the differences observed between the common streptococci of the saliva and those of the fæces may find their explanation along these lines, since it must be presumed that the fæcal streptococci originally reach the intestine by descending from the mouth. The change apparently is a very gradual one, for Andrewes and Horder state (1906, 7), with regard to the reactions of these streptococcal types, that "*S. salivarius* passes by insensible gradations into *S. fæcalis.*"

Further, it may be regarded as at least possible that the streptococci present in saliva actually acquire the *S. salivarius* type (or types) during and owing to their residence in the mouth. If this is not the case, how does so comparatively great a degree of uniformity of reactions as is described come to occur among the streptococci found in an open cavity like the mouth? Or again, how comes it that the common streptococcus of the air does not appear among the types stated to be most common in the saliva?

The fact that, although the total number of types described in the saliva and in fæces is very considerable (in saliva 48 and in fæces 40), yet the great majority of all the streptococci found have been shown to fall into a few main groups, itself suggests the possibility that a persistent adaptation goes on in these situations, as a result of which the streptococci entering the mouth or reaching the intestine constantly tend to assume particular series of reactions.

The findings in a number of my own earlier experiments led me to suggest the possibility that in particular surroundings streptococci tend to assume particular types in relation to Gordon's tests—in fact, that the reactions differentiate particular environments rather than particular varieties of streptococci. The evidence, which is somewhat striking and undoubtedly suggestive, is shown in Tables V, VI, VII, and VIII.

Table V.—Showing the Reactions of certain Streptococci in 1910 after Continuous Cultivation in Stab Agar for Two Years.

Six of the Streptococci agree in every reaction but one.

Streptococcus.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.
L	0	0	+	0	0	+	0
P	0	0	+	0	0	+	0
V	0	0	+	0	0	+	0
S	0	+	+	0	0	+	0
M	0	+	+	0	0	+	0
H	0	+	+	0	0	+	0
G	+	+	+	+	0	+	0

Table VI.—Showing the Reactions of some of the Streptococci from Table V after Eight Weeks' (in the case of P Nine Weeks') Cultivation in Inulin Medium.

Three are identical, and one other differs only in respect of one reaction.

Streptococcus.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.
L	+	+	+	+	+	+	+
S	+	+	+	+	+	+	+
G	+	+	+	+	+	+	+
M	0	+	+	+	+	+	+
P	+	0	+	0	+	+	0

Table VII.—Showing the Reactions of the Streptococci of Table VI after their return for Three Months to Cultivation in Stab Agar.

Three are identical, and show the same reactions as L, P, V, *from agar in* 1910, *and* G *only differs as regards the saccharose reaction.*

Streptococcus.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.
L	0	0	+	0	0	+	0
P	0	0	+	0	0	+	0
S	0	0	+	0	0	+	0
G	0	+	+	0	0	+	0
M	0	+	+	+	+	+	+

Table VIII.—Showing the Reactions of some of the Streptococci from Table V after Four Months' Cultivation in Mannite Medium.

Three are identical, and V *differs only in regard to the clotting of milk.*

Streptococcus.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.
L	0	0	+	0	0	+	0
P	0	0	+	0	0	+	0
M	0	0	+	0	0	+	0
V	+	0	+	0	0	+	0
S	0	+	0	0	0	0	0

The 1912 reactions of the streptococci L, P, G, M, V (all of human derivation) tested after 18 months' continuous cultivation in stab agar further support the hypothesis, since with the exception of two differences in the saccharose reaction they are seen in Table IX to present an absolute identity.

Table IX.—Showing the Reactions of some of the Streptococci from Table V after 18 Months' further Cultivation in Stab Agar.

Three are identical, and the other two differ only in regard to the saccharose reaction. The reason why the reactions differ from those obtained with agar cultures in 1910 is unknown. It is not known, for example, whether the agar has undergone any change. The leading reactions are the same as those of P from agar in 1908.

Streptococcus.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.
L	+	0	+	+	0	+	0
P	+	0	+	+	0	+	0
M	+	0	+	+	0	+	0
G	+	+	+	+	0	+	0
V	+	+	+	+	0	+	0

Accordingly I conclude that the tests may assist in the determination of the source and origin of particular strains of streptococci met with. It is, however, necessary to speak with a good deal of reserve upon this point because at present we do not possess enough exact evidence as to the length of time that streptococci can retain particular characters in respect of their test reactions, nor as to the length of time and other conditions necessary for the impression of new characters upon them. Meanwhile it is probably true that in certain cases the source of particular streptococci may safely be deduced from their reactions in the test media, and that, as Andrewes and Horder clearly pointed out, "a streptococcus from the mouth fermenting only saccharose, salicin, and coniferin, non-pathogenic, and incapable of growth on gelatin at 20° C. may with some confidence be referred to inhaled horse-dung." The question is clearly worth pursuing further.

On the other hand, in at any rate a considerable proportion of cases, the results obtained by applying Gordon's tests must at present remain inconclusive owing to the lack of sharpness and definition in the demarcation of the different streptococcal types to which attention has already been called. Thus, as Houston (1905, 9) pointed out in discussing the possibility of recognising fæcal contamination by means of streptococcal tests, "the fact of there being so many groups" of fæcal streptococci "unfortunately suggests the likelihood of streptococci isolated from various and perhaps unobjectional sources falling almost necessarily under one or other of the 40 groups." And again, "unfortunately, with so many groups to select from, a particular streptococcus . . . . derived from a non-fæcal source could hardly fail to fall under one or another of the 40 groups of fæcal streptococci."

This is perhaps to state the case more strongly than necessary, since the 40 fæcal groups present after all only a fraction of the 512 different series of reactions theoretically possible with Gordon's nine tests. Nevertheless, it is to be remembered on the other side that the *à priori* probability of the appearance of the different series is by no means equal, since all the sugars are not equally easily fermented.

It is of interest to note in this connection that if the varieties of streptococci already described by different observers who have used the tests were to be accepted as fixed and definite independent organisms more than 100 different kinds of streptococci have probably already been identified.

Conclusions.

1. There is at present no proof of the existence of more than one kind of streptococcus pathogenetic for man.

2. The reactions obtained with streptococci in Gordon's media are largely dependent on the character of the environment in which the organisms have previously been growing.

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The Specific Conductivity of Solutions of Oxyhæmoglobin.

By Prof. G. N. STEWART.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received May 23,—Read June 27, 1912.)

(From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, U.S.A.)

When preparing a paper on the mechanism of hæmolysis two or three years ago* my attention was accidentally called to a statement in a paper by the late Prof. A. Gamgee in the 'Proceedings' of the Royal Society,† that "although solutions of oxyhæmoglobin possess a low conductivity this is very much higher than has been found in the previous observations of Stewart." In a note appended to my paper I suggested that this could only mean "that either his (Gamgee's) oxyhæmoglobin or his distilled water was less thoroughly freed from electrolytes than mine. In observations of this kind the error must appear as too high and not as too low a conductivity."

Prof. Gamgee having laid stress on the purity of his distilled water and oxyhæmoglobin, this result seemed very puzzling, all the more as my object in determining the conductivity of some specimens of oxyhæmoglobin‡ was merely to control their suitability for addition to blood in the determination of the relative volume of corpuscles and plasma by a colorimetric method described in the paper, and no such effort has been made to carry the exclusion of foreign electrolytes to the practically possible limit as would have been deemed indispensable had the conductivity of hæmoglobin been investigated for its own sake.

Having tried in vain to procure a full report of the Croonian Lecture in which Prof. Gamgee's research was embodied, I had almost given up hope of being able to solve the puzzle. A few weeks ago, however, on looking again at the abstract in the 'Proceedings' of the Society, the probable explanation of Prof. Gamgee's mistake occurred to me. My experiments on the conductivity of animal liquids were begun more than 20 years ago,§ and specific conductivity in all the papers was expressed, as was usual at that time, in terms of that of mercury, the conductivity of a column of mercury 106·3 cm. in length and 1 sq. mm. in cross-section being taken as unity. Since the resistance of a column of mercury of these dimensions at 0° C. is an ohm, the unit of conductivity was spoken of, for the sake of brevity, as a reciprocal ohm. This unit is 10^4 times smaller than the unit now employed. All my results must therefore be multiplied by 10^4 to express them in the new unit. The further factor 1·063 by which it is necessary to multiply conductivities expressed as the reciprocals of resistances measured in Siemens units must not be applied to my results. It is perhaps worth while stating this, as Höber,* in transposing some of my results to the new unit, multiplies not only by 10^4 but also by 1·063. This is erroneous.

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* 'Journ. Pharm. and Exper. Therap.,' 1909, vol. 1, p. 49.

† 1902, vol. 70, p. 82.

‡ 'Journ. Physiol.,' 1899, vol. 24, p. 356.

§ 'Studies from the Physiological Laboratory of the Owens College, Victoria University, Manchester,' 1891, p. 124; 'Physiol. Soc. Proc.,' Nov. 8, 1890; 'Journ. Physiol.,' vol. 11, p. 15; Boston Society of Medical Sciences, June 15, 1897; 'Journ. Physiol.,' 1897, vol. 22, p. 159; *ibid.*, 1899, vol. 24, p. 211, etc.

The specific conductivity of a 3·5-per-cent. solution of oxyhæmoglobin at 5° C. was given by me† as $0·90 \times 10^{-8}$, and that of a 1·2-per-cent. solution‡ as $0·53 \times 10^{-8}$. In the new unit these numbers would be $0·90 \times 10^{-4}$ and $0·53 \times 10^{-4}$, *i.e.*, 9×10^{-5} and $5·3 \times 10^{-5}$ respectively. Now for a 3·07-per-cent. solution of oxyhæmoglobin Prof. Gamgee gives as the conductivity at 0° C. $2·626 \times 10^{-5}$, and at 18° C. $4·432 \times 10^{-5}$. For a 2·23-per-cent. solution of oxyhæmoglobin he gives at 0° C. $2·23 \times 10^{-5}$, and at 18° C. $3·25 \times 10^{-5}$. Although he does not use the symbol K, and merely states that his numbers are "expressed in reciprocal ohms," it may be assumed that he meant to give them in the new unit. Accordingly, it would appear that the conductivities observed by him, instead of being very much higher than mine, are really lower, which is perfectly intelligible.

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* 'Physikalische Chemie der Zelle und der Gewebe,' 1st edit., p. 132.

† 'Journ. Physiol.,' 1899, vol. 24, p. 358.

‡ *Ibid.*, p. 359.

The Chemical Action on Glucose of a Variety of Bacillus coli communis (*Escherich*), *obtained by Cultivation in Presence of a Chloroacetate.* (*Preliminary Notice.*)

By A. Harden, F.R.S., and W. J. Penfold.

(Received May 18,—Read June 20, 1912.)

(Biochemical Department of the Lister Institute.)

Penfold has shown (1) that when *Bacillus coli communis* (Escherich) is plated out on nutrient agar containing 0·5 per cent. sodium chloroacetate, a large number of small colonies and a few large ones are produced. The cultures obtained from some, but not all, of the large colonies were found to have lost the power of producing gas from glucose when grown in glucose peptone water in a test tube provided with a Durham gas tube. Under the same conditions these same organisms still produced gas from mannitol, but in diminished proportion. Several other intestinal organisms have since been found by Harden to behave in a similar manner on chloroacetate agar, whereas *B. lactis aërogenes*, which produces a different type of decomposition of sugar, has hitherto proved resistant to this method of selection. In order to ascertain what other modification the chemical action of the original strain had undergone, comparative quantitative experiments were carried out with the original and the selected organisms.

Product.	Percentage of sugar used.		Carbon atoms per molecule of glucose.		Harden's theory for *B. coli communis.* Carbon atoms.
	Normal *B. coli.*	Selected *B. coli.*	Normal *B. coli.*	Selected *B. coli.*	
Alcohol	17·22	5·5	1·35	0·43	1
Acetic acid	20·60	10·02	1·24	0·60	1
Formic acid	2·55	3·53	0·10 } 0·81	0·14 } 0·22	1
Carbon dioxide	17·30	2·00	0·71	0·08	
Lactic acid	40·60	77·6	2·43	4·66	3
Succinic acid	4·80	None	0·29	None	
	93·07	98·65	6·12	5·91	6
H (as gas) c.c. per grm. of sugar	80·6	12·6			
H as gas + H equivalent of formic acid	92·9	29·8			

The medium employed in both cases contained 10 grm. peptone and 20 grm. glucose per litre, 10 grm. of chalk being added per litre. The flasks were connected with the gas-collecting apparatus described by Harden, Thompson, and Young (2), and the air of the flask replaced by nitrogen before incubation. The estimations were made as previously described (3). The results of two such experiments are given in the table on p. 415, the products being expressed both in percentage of the sugar used and as the number of carbon atoms of the glucose molecule to which they correspond.

These results must be regarded as preliminary, but are of sufficient interest to justify some remark at the present stage of the work.

In the first place, it is to be noted that, although the selected organism gave no gas at all when tested by the Durham tube method, it yielded about 12·6 c.c. of hydrogen per gramme of sugar when grown anaërobically in presence of chalk as against 80·6 given by the original organism. Similarly the amount of gaseous CO_2 formed from the sugar was found to be 10·3 c.c. per gramme, but as this number is the difference of two large volumes (the total gaseous CO_2 + CO_2 dissolved in the medium − CO_2 liberated by the acids formed) no great accuracy attaches to it. The reason of this different behaviour is not understood and is at present being investigated.

Apart from this, an examination of the results shows that the main difference between the actions of the two bacteria is to be found in the increased proportion of lactic acid, and the correspondingly diminished proportion of the other products, formed by the selected organism.

Harden (3) has previously shown that the action of organisms closely allied to *B. coli communis* is roughly represented by the equation

$$(1)\quad 2C_6H_{12}O_6 + H_2O = 2C_3H_6O_3 + C_2H_6O + C_2H_4O_2 + 2CO_2 + 2H_2,$$

which requires the proportions of products shown in the last column of the table. Actually there is always less CO_2 than this produced, and with the strain of normal *B. coli communis* here employed there is also somewhat more alcohol and acetic acid and less lactic acid formed. The relations between the products of the selected organism appear, however, to be quite incompatible with this equation.

The explanation which suggests itself is that the products observed as the result of action of the normal organism are formed by three independent enzymes. One of these converts sugar into lactic acid :

$$(2)\quad C_6H_{12}O_6 = 2C_3H_6O_3.$$

The other probably produces alcohol, acetic acid, and formic acid, and the last of these is in all probability decomposed by a third enzyme into carbon dioxide and hydrogen :

$$(3)\quad C_6H_{12}O_6+H_2O = C_2H_4O_2+C_2H_6O+2HCO_2H$$
$$= C_2H_4O_2+C_2H_6O+2CO_2+2H_2.$$

These enzymes may occur in different proportions in different individual organisms, and the process of selection would then result in the survival of an organism containing a large proportion of the lactic acid enzyme and a small proportion of that producing alcohol, acetic acid, and formic acid. It may be noted that the selected organism still retains the power of decomposing formic acid into CO_2 and H_2, and, moreover, its products do not contain more of this acid than those of the normal bacillus. The absence of gas formation, therefore, is not due to an accumulation of formic acid. It is thus seen that the change produced in the organism does not really cause its chemical action to approximate to that of *B. typhosus*, an organism which also yields no gas, but differs from *B. coli* by producing a large amount of formic acid.

The foregoing numbers are not sufficiently accurate to decide with certainty whether the relative proportions of alcohol, acetic acid, and formic acid produced by the selected organism are the same as those formed by the original bacillus. Taking the alcohol as standard, the carbon as formic acid (including gaseous CO_2) should be 0·27, instead of 0·22 observed, and that as acetic acid 0·41 instead of 0·6. The deviation in the last case may be due to the difficulty of effecting the quantitative separation of acetic acid from a large proportion of lactic acid.

Further experiments may be expected to throw light on these points.

If, however, the foregoing explanation be found correct, the study of the chemical action of these selected organisms should form a valuable means of investigation into the nature of the various processes of fermentation effected by bacteria, and the subject is being prosecuted from this point of view.

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(2) 'Biochemical Journal,' 1910, vol. 5, p. 230.
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The Action of Enzymes on Hexosephosphate.

By Victor J. Harding.

(Communicated by A. Harden, F.R.S. Received May 21,—Read June 20, 1912.)

(Biochemical Department of the Lister Institute.)

According to the theory advanced by Harden and Young,* the presence of phosphate is essential for the alcoholic fermentation of sugar by yeast-juice. In the presence of the fermenting complex, the phosphate and sugar react together, with the simultaneous production of equivalent quantities of carbon dioxide, alcohol, and hexosephosphate. The hexosephosphate is then hydrolysed by an enzyme present in yeast-juice, with formation of a hexose and free phosphate, and the latter then again undergoes the first reaction with more sugar. The phosphate thus repeatedly passes through a cycle which may be represented by the following equations :—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O$,

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4$.

The normal rate of fermentation of excess of sugar by active yeast-juice is therefore dependent upon the rate at which phosphate is set free from the hexosephosphate, and any acceleration of this reaction would increase the rate of fermentation of the sugar. Thus the addition to yeast-juice and sugar of a hexosephosphatase, that is, an enzyme capable of hydrolysing hexosephosphate, would be expected to bring about this result, and the following experiments on the action of various enzyme preparations on sodium hexosephosphate have been carried out primarily with the object of finding such an enzyme.

As a result, it was found that enzymes of very different origin (Ricinus lipase and almond emulsin) were capable of hydrolysing hexosephosphate, but the effect of adding these to fermenting yeast-juice has not yet been ascertained.

Preparation of Hexosephosphoric Acid.—The solution of hexosephosphoric acid used in these experiments was prepared as described by Young,† by decomposing the lead salt with hydrogen sulphide. The solution of free hexosephosphoric acid was made neutral to litmus by a known volume of sodium hydrate solution, and such a neutral solution was used in all the experiments. The amount of hexosephosphoric acid was previously determined by titration of 1 c.c. with N/10 KOH, phenolphthalein as indicator. In all experiments the term hexosephosphate is used to designate the solution neutral to litmus. The extent of hydrolysis was in each case ascertained by precipitating the liberated phosphate with magnesium citrate mixture, igniting and weighing as magnesium pyrophosphate. All results, as well as the concentration of the original hexosephosphate solutions, are expressed in terms of magnesium pyrophosphate.

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* 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 299.

† 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 531.

The following enzyme preparations were employed :—

(*a*) lipase from castor oil seeds; (*b*) autolysed ox pancreas; (*c*) emulsin; (*d*) zymin extract; (*e*) autolysed yeast-juice.

(*a*) *Lipase from Castor Oil Seeds* (Ricinus communis).—This was prepared by extracting the freshly ground seeds of *Ricinus communis* with ether until fat free, and using the residue as the lipase preparation. It was found to be highly active.

The results of three experiments are given in the accompanying table, which refers in each case to the following set of mixtures :—

A. 0·3 grm. lipase + 3 c.c. N/5 acetic acid + 15 c.c. water.
B. „ „ „ + 15 c.c. hexosephosphate.
C. Same as B, but boiled before incubation.

The experiments were carried out at 25° in presence of toluene (0·5 c.c.), and different hexosephosphate solutions were employed in each set of experiments.

No.	Original hexosephosphate as $Mg_2P_2O_7$.	Time of incubation in hours.	Free phosphate, grm. $Mg_2P_2O_7$.			Phosphate liberated, B – C.
			A.	B.	C.	
1	0·5835	24	0	0·1259	0·0428	0·0831
		48	0	0·1836	0·0320	0·1516
2	0·5665	120	0	0·2746	0·0416	0·2330
3	0·6274	240	0	0·3655	0·0359	0·3296

It is seen that the preparation had a considerable hydrolytic action on the hexosephosphate.

(*b*) *Ox Pancreas.*—The fresh ox pancreas, freed as far as possible from connective tissue, was allowed to undergo autolysis for four days under toluene at 37°. The liquid was then filtered through a coarse muslin cloth to remove any undigested pancreas, and the filtrate used in the experiments.

The following mixtures were incubated for 48 hours at 37° in presence of toluene, and the free phosphate estimated as before:—

A. 5 c.c. autolysed ox pancreas + 15 c.c. water.
B. „ „ + 15 c.c. hexosephosphate.
C. The same as B, but the pancreas mixture employed was boiled.

Original hexosephosphate = 0·5351 grm. $Mg_2P_2O_7$.
Free phosphate in A ...... = 0·0 „
„ B ...... = 0·0915 „
„ C ...... = 0·0814 „
Phosphate liberated ...... = 0·0101 „

Practically no action had taken place.

(*c*) *Emulsin.*—The emulsin was prepared as described by H. E. and E. F. Armstrong and Horton.*

The experiments were carried out at 37° in presence of toluene, the following mixtures being employed:—

A. 10 c.c. emulsin solution + 15 c.c. water.
B. „ „ + 15 c.c. hexosephosphate.
C. Same as B, but boiled emulsin employed.

Original hexosephosphate = 0·6274 grm. $Mg_2P_2O_7$.

	After	
	48 hours.	144 hours.
Free phosphate in A	0·0	0·0
„ „ B	0·1750	0·3064
„ „ C	0·0342	0·0563
Phosphate set free by enzyme ...	0·1408	0·2501

Emulsin has, therefore, a considerable hydrolytic action on hexosephosphate.

(*d*) *Zymin Extract.*—10 grm. of "zymin" preparation were digested four days with 30 c.c. water at 25°. The resulting mixture was filtered and used in the experiment. It was found to contain considerable amounts of phosphate.

An aqueous extract of zymin (yeast treated with acetone, was found only to have a slight action on hexosephosphate. This is seen from the following experiment carried out at 25° in presence of toluene, the mixture being incubated for three days:—

---

* 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 324.

A. 5 c.c. zymin extract + 15 c.c. water.
B. „ „ + 15 c.c. hexosephosphate.
C. Same as B, but zymin extract boiled.

Original hexosephosphate............... = 0·6274 grm. $Mg_2P_2O_7$.
After incubation, free phosphate in—
A = 0·0625 „
B = 0·1618 „
C = 0·0910 „
Phosphate set free by enzyme......... = 0·0708 „

(*e*) *Autolysed Yeast-juice.*—A sample of yeast-juice which had been allowed to remain at 0° for a fortnight was used. It was found to be incapable of fermenting glucose.

The following mixtures were incubated at 25° for 24 hours in presence of toluene, and the free phosphate estimated:—

A. Autolysed yeast-juice 25 c.c. + 10 c.c. water.
B. ” ” + 10 c.c. hexosephosphate.
C. As B, but boiled yeast-juice employed.

Original hexosephosphate .... = 0·3776 grm. $Mg_2P_2O_7$.
After incubation, free phosphate in—
A = 0·1389 ”
B = 0·2232 ”
C = 0·1409 ”
Phosphate set free by enzyme......... = 0·0823 ”

Hexosephosphate is thus hydrolysed to some extent by autolysed yeast-juice. This is in agreement with the experiments of Harden and Young, who showed that in actively fermenting yeast-juice most of the phosphate was present in the combined form, but was gradually set free by an enzyme hexosephosphatase, after the juice had lost its fermentative activity.*

An attempt was made to ascertain if hexosephosphatase could be isolated from the autolysed yeast-juice: 50 c.c. of autolysed yeast-juice were added with constant stirring to a mixture of 400 c.c. alcohol and 400 c.c. ether. The white precipitate was filtered immediately at the pump, washed once with ether, and air dried. A very small amount of a brownish sticky precipitate remained. This was dissolved in 50 c.c. of water and allowed to stand overnight with a little toluene. It was then filtered through muslin, and the solution thus obtained examined as to its hydrolytic properties towards hexosphosphate.

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* 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 299.

The mixtures A, B, C were incubated with toluene at 25° for 24 hours :—

A. 10 c.c. above solution + 15 c.c. water.
B. „ „ + 15 c.c. hexosephosphate.
C. As B, but enzyme solution previously boiled.

Original hexosephosphate .... = 0·5665 grm. $Mg_2P_2O_7$.
After incubation, free phosphate in—
A = 0·0494 „
B = 0·1268 „
C = 0·0755 „
Phosphate set free by enzyme .... = 0·0513 „

The hexosephosphatase, therefore, can be precipitated by means of alcohol and ether.

Summary.

(*a*) Ricinus lipase and emulsin possess a slow hydrolytic action on hexosephosphate.

(*b*) Autolysed ox pancreas is almost without action upon hexosephosphate.

(*c*) "Zymin" extract hydrolyses hexosephosphate slowly.

(*d*) Autolysed yeast-juice possesses a marked action on hexosephosphate.

(*e*) An alcohol-ether precipitation of autolysed yeast-juice possesses considerable activity towards hexosephosphate.

The Morphology of the Trypanosome Causing Disease in Man in Nyasaland.

By Colonel Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; Dr. J. B. Davey, Nyasaland Medical Staff; and Lady Bruce, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1911—12.)

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[Plates 11 and 12.]

Introduction.

This species, like *Trypanosoma brucei* and *T. gambiense*, is characterised by showing two distinct forms—the long and slender, and the short and stumpy. These are not sharply divided from one another, but are connected by intermediate forms, so as to form an unbroken series, or curve, from the shortest to the longest.

This strain of the trypanosome was obtained from a native woman suffering from "Kaodzera," the so-called Sleeping Sickness of Nyasaland.

A. *Living, Unstained.*

The movements of the Nyasaland trypanosome resemble *T. brucei* and *T. gambiense* in being non-translatory either in fresh blood or in dilutions with citrate solution. Under dark background illumination, however, with a higher degree of dilution, some translatory movement is seen, the trypanosomes swimming slowly across the field.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as previously described in the "Proceedings."*

Length.—The following table gives the length of this trypanosome as found in man, monkeys, goats, sheep, dogs, guinea-pigs, and white rats—1220 trypanosomes in all. From this it will be seen that it measures, on an average, rather more than *T. brucei* or *T. gambiense*, but the difference is so small as to be probably of no value as a means of separating these species.

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1.9.10	—	Man (P.R.)	Osmic acid	Giemsa	23·6	28·0	18·0
1.9.10	—	„	„	„	24·3	30·0	18·0
1.9.10	—	„	„	„	23·9	29·0	20·0
29.1.12	24	Monkey	„	„	22·1	30·0	17·0
6.2.12	24	„	„	„	18·0	21·0	16·0
8.2.12	24	„	„	„	19·9	29·0	16·0
15.2.12	24	„	„	„	20·2	23·0	17·0
19.2.12	234	„	„	„	23·0	31·0	18·0
15.2.12	142	Goat	„	„	24·3	28·0	21·0
9.2.12	163	Sheep	„	„	25·0	29·0	20·0
9.2.12	163	„	„	„	25·1	29·0	22·0
17.2.12	164	„	„	„	24·6	29·0	21·0
19.1.12	11	Dog	„	„	28·3	34·0	23·0
25.1.12	14	„	„	„	25·9	32·0	17·0
29.1.12	14	„	„	„	22·7	30·0	16·0
5.2.12	111	„	„	„	23·4	28·0	18·0
6.2.12	111	„	„	„	27·1	33·0	18·0
7.2.12	111	„	„	„	25·1	31·0	17·0
8.2.12	111	„	„	„	24·6	31·0	18·0
12.2.12	111	„	„	„	23·7	30·0	17·0
9.2.12	111	„	„	„	24·5	29·0	15·0
13.2.12	111	„	„	„	20·3	27·0	16·0
15.2.12	111	„	„	„	24·1	32·0	16·0
14.2.12	111	„	„	„	23·2	29·0	17·0
14.2.12	111	„	„	„	22·5	33·0	17·0
19.1.12	13	Guinea-pig	„	„	19·0	28·0	14·0
25.1.12	13	„	„	„	24·6	36·0	16·0
29.1.12	13	„	„	„	22·2	28·0	15·0
12.2.12	13	„	„	„	23·1	29·0	16·0
19.2.12	165	„	„	„	25·0	30·0	16·0
15.2.12	13	„	„	„	21·7	28·0	16·0
19.12.11	38	Rat	„	„	26·6	33·0	20·0
22.1.12	38	„	„	„	27·7	33·0	18·0
22.1.12	37	„	„	„	26·7	33·0	17·0
29.1.12	36	„	„	„	28·2	31·0	23·0
1.2.12	36	„	„	„	26·4	33·0	22·0
6.2.12	36	„	„	„	28·3	34·0	18·0
6.2.12	36	„	„	„	27·2	33·0	18·0
1.2.12	36	„	„	„	27·2	35·0	21·0
6.2.12	36	„	„	„	28·2	31·0	19·0
20.2.12	236	„	„	„	21·6	30·0	18·0
20.2.12	236	„	„	„	22·7	29·0	18·0
22.2.12	235	„	„	„	25·5	29·0	18·0
21.2.12	235	„	„	„	25·1	29·0	19·0
21.2.12	236	„	„	„	25·5	30·0	19·0
21.2.12	236	„	„	„	25·1	34·0	19·0
22.2.12	235	„	„	„	24·8	31·0	19·0
22.2.12	236	„	„	„	24·7	33·0	18·0
22.2.12	236	„	„	„	24·0	34·0	19·0
22.2.12	235	„	„	„	23·5	28·0	19·0
23.2.12	235	„	„	„	24·0	33·0	18·0
23.2.12	235	„	„	„	25·2	32·0	19·0
23.2.12	236	„	„	„	25·5	31·0	19·0
24.2.12	235	„	„	„	24·4	29·0	19·0
1.2.12	36	„	„	„	28·7	34·0	19·0

Table I—*continued.*

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
23.2.12	236	Rat	Osmic acid	Giemsa	24·5	34·0	18·0
24.2.12	236	"	"	"	20·9	29·0	18·0
24.2.12	235	"	"	"	25·4	32·0	20·0
24.2.12	236	"	"	"	22·2	30·0	18·0
26.2.12	235	"	"	"	21·8	31·0	16·0
26.2.12	236	"	"	"	21·5	30·0	18·0
					24·1	36·0	14·0

The average length of the trypanosome of the human trypanosome disease of Nyasaland in man and other species of animals, taken from Table I, is as follows:—

Table II.

Species of animal.	No. of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Man	60	23·9	30·0	18·0
Monkey*	100	20·6	31·0	16·0
Goat	20	24·3	28·0	21·0
Sheep	60	24·9	29·0	22·0
Dog	260	24·2	34·0	15·0
Guinea-pig	120	22·6	36·0	14·0
Rat	600	25·1	35·0	16·0

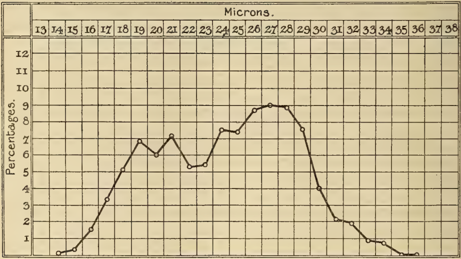
* Probably *Cercopithecus pygerythus*, the common grey, black-faced monkey; native name "Pusi."

Table III.—Distribution in respect to Length of 1220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Animal.	In microns.																							Average length.
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	
Man	—	—	—	—	—	—	1	4	2	—	4	3	4	1	—	1	—	—	—	—	—	—	—	23·6
„	—	—	—	—	1	—	2	—	3	3	3	3	3	1	1	—	—	—	—	—	—	—	—	24·3
„	—	—	—	—	1	—	1	4	—	2	2	2	2	2	3	—	1	—	—	—	—	—	—	23·9
Monkey	—	—	—	3	—	4	—	2	3	2	2	—	1	—	1	1	1	—	—	—	—	—	—	22·1
„	—	—	2	6	5	5	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18·0
„	—	—	1	5	2	4	2	1	1	1	—	2	—	—	—	1	—	—	—	—	—	—	—	19·9
„	—	—	—	1	3	3	3	6	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	20·2
„	—	—	—	—	1	2	3	3	—	3	3	—	1	2	—	1	—	1	—	—	—	—	—	23·0
Goat	—	—	—	—	—	—	—	1	2	4	5	3	2	1	2	—	—	—	—	—	—	—	—	24·3
Sheep	—	—	—	—	—	—	1	1	1	1	3	4	4	2	2	1	—	—	—	—	—	—	—	25·0
„	—	—	—	—	—	—	—	—	3	2	4	2	2	4	2	1	—	—	—	—	—	—	—	25·9
„	—	—	—	—	—	—	—	2	—	4	4	6	—	2	—	2	—	—	—	—	—	—	—	24·6
Dog	—	—	—	—	—	—	—	—	—	2	—	2	2	—	3	5	1	2	2	—	1	—	—	28·3
„	—	—	—	1	—	—	—	—	—	—	4	3	2	7	1	1	—	—	1	—	—	—	—	25·1
„	—	—	1	1	—	4	2	1	1	1	1	3	—	2	1	1	1	—	—	—	—	—	—	22·7
„	—	—	—	—	1	2	3	1	2	—	1	3	2	3	2	—	—	—	—	—	—	—	—	23·4
„	—	—	—	—	1	1	—	—	1	—	1	—	4	—	4	2	3	1	1	1	—	—	—	27·1
„	—	—	—	1	—	2	1	—	2	—	—	3	—	6	1	2	1	1	—	—	—	—	—	25·1
„	—	—	—	—	1	3	—	—	2	2	2	1	—	4	2	1	1	1	—	—	—	—	—	24·6
„	—	—	—	2	2	1	—	1	—	1	2	1	6	1	2	—	1	—	—	—	—	—	—	23·7
„	—	1	—	2	—	—	—	2	—	—	2	3	2	3	2	3	—	—	—	—	—	—	—	24·5
„	—	—	1	3	7	2	—	—	—	2	—	1	3	1	—	—	—	—	—	—	—	—	—	20·3
„	—	—	2	—	2	—	—	—	—	2	3	1	6	1	2	—	—	—	1	—	—	—	—	24·1
„	—	—	—	1	3	1	—	2	2	—	3	2	1	1	2	2	—	—	—	—	—	—	—	23·2
„	—	—	—	4	3	1	1	1	—	1	2	—	1	2	2	1	—	—	—	1	—	—	—	22·5
Guinea-pig	1	2	4	1	2	1	1	4	2	—	1	—	—	—	1	—	—	—	—	—	—	—	—	19·0
„	—	—	2	1	1	—	1	—	3	—	—	2	2	2	1	1	3	—	—	—	—	—	1	24·6
„	—	1	—	4	3	1	—	—	—	—	2	1	2	4	2	—	—	—	—	—	—	—	—	22·2
„	—	—	1	4	1	2	—	1	—	—	—	2	1	1	5	2	—	—	—	—	—	—	—	23·1
„	—	—	2	—	1	—	—	—	—	—	1	4	5	3	2	1	1	—	—	—	—	—	—	25·0
„	—	—	2	1	1	2	1	3	3	—	2	1	1	2	1	—	—	—	—	—	—	—	—	21·7

Rat	—	—	—	—	—	—	1	1	2	—	—	3	2	4	1	2	1	1	—	2	—	—	—	26·6
,, ...	—	—	—	—	1	—	—	1	—	—	1	—	1	3	5	3	1	2	1	1	—	—	—	27·7
,, ...	—	—	—	1	1	—	1	2	—	—	—	1	1	1	4	1	3	—	3	1	—	—	—	26·7
,, ...	—	—	—	—	—	—	—	—	—	1	1	1	1	1	3	6	5	1	—	—	—	—	—	28·2
,, ...	—	—	—	—	—	—	—	—	2	2	2	1	2	4	2	3	—	1	—	1	—	—	—	26·4
,, ...	—	—	—	—	1	2	—	—	—	—	—	—	1	—	3	4	3	2	2	—	2	—	—	28·3
,, ...	—	—	—	—	1	1	2	—	—	—	1	—	1	2	2	2	4	—	3	1	—	—	—	27·2
,, ...	—	—	—	—	—	—	—	2	—	—	2	1	3	2	4	2	1	1	1	—	—	1	—	27·2
,, ...	—	—	—	—	—	1	—	—	—	—	1	—	4	1	—	3	6	4	—	—	—	—	—	28·2
,, ...	—	—	—	—	1	1	7	4	1	3	1	—	—	1	—	—	1	—	—	—	—	—	—	21·6
,, ...	—	—	—	—	2	4	4	—	—	1	1	2	—	3	2	1	—	—	—	—	—	—	—	22·7
,, ...	—	—	—	—	1	—	2	1	—	1	—	3	2	3	2	5	—	—	—	—	—	—	—	25·5
,, ...	—	—	—	—	—	1	1	3	2	—	1	2	—	2	4	4	—	—	—	—	—	—	—	25·1
,, ...	—	—	—	—	—	1	—	2	—	1	1	2	7	2	—	3	1	—	—	—	—	—	—	25·5
,, ...	—	—	—	—	—	3	1	—	2	3	—	—	3	1	2	3	1	—	—	—	1	—	—	25·1
,, ...	—	—	—	—	—	1	2	1	2	2	3	—	1	1	5	—	1	1	—	—	—	—	—	24·8
,, ...	—	—	—	—	1	1	—	3	1	3	2	2	1	1	1	—	2	1	—	1	—	—	—	24·7
,, ...	—	—	—	—	—	1	1	7	1	1	—	2	3	1	—	1	—	—	1	—	1	—	—	24·0
,, ...	—	—	—	—	—	1	2	2	3	3	3	—	2	1	3	—	—	—	—	—	—	—	—	23·5
,, ...	—	—	—	—	1	—	3	1	4	2	3	1	—	1	—	1	—	2	—	1	—	—	—	24·0
,, ...	—	—	—	—	—	1	1	3	1	1	2	—	2	2	3	2	1	—	1	—	—	—	—	25·2
,, ...	—	—	—	—	—	1	2	2	—	—	2	2	2	2	2	2	1	2	—	—	—	—	—	25·5
,, ...	—	—	—	—	—	1	2	2	3	—	—	2	4	2	2	2	—	—	—	—	—	—	—	24·4
,, ...	—	—	—	—	—	1	—	—	—	—	1	3	3	1	1	—	—	1	5	3	1	—	—	28·7
,, ...	—	—	—	—	1	1	5	—	—	3	—	2	—	2	1	2	1	1	—	—	1	—	—	24·5
,, ...	—	—	—	—	4	3	7	—	2	1	—	1	—	—	—	2	—	—	—	—	—	—	—	20·9
,, ...	—	—	—	—	—	—	1	3	1	—	3	1	1	5	3	1	—	—	1	—	—	—	—	25·4
,, ...	—	—	—	—	1	6	2	3	—	2	1	1	—	1	1	1	1	—	—	—	—	—	—	22·2
,, ...	—	—	1	—	2	6	—	3	—	1	3	—	1	1	—	1	—	1	—	—	—	—	—	21·8
,, ...	—	—	—	—	3	2	5	3	3	—	1	—	—	1	1	—	1	—	—	—	—	—	—	21·5
Total	1	4	19	42	63	81	75	91	65	66	93	91	107	110	104	87	49	27	23	13	7	1	1	
Percentages ...	0·1	0·3	1·5	3·4	5·1	6·8	6·0	7·3	5·3	5·4	7·5	7·4	8·7	9·0	8·5	7·5	4·0	2·2	1·9	1·0	0·6	0·1	0·1	

Chart 1.—Chart giving Curve representing the Distribution, by Percentages, in respect to Length, of 1220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.



This curve is made up of measurements from 60 specimens of trypanosomes taken from man, 100 from the monkey, 20 from the goat, 60 from the sheep, 260 from the dog, 120 from the guinea-pig, and 600 from the rat.

Table IV.—Measurements giving the Average Distance from the Posterior Extremity to Micronucleus, Micronucleus to Nucleus, etc., in 1220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Posterior extremity to micronucleus.	Micronucleus to nucleus.	Diameter of nucleus.	Nucleus to anterior extremity.	Flagellum.
Short and stumpy (14 to 21 microns), 620 individuals.				
1·4	4·4	2·7	10·0	0·6
Intermediate (22 to 24 microns), 224 individuals.				
1·6	5·6	2·9	9·3	3·7
Long and slender (25 to 36 microns), 376 individuals.				
2·0	6·3	3·1	10·4	5·8

The measurements are made along the curve of the long axis of the body of the trypanosome, and therefore if an oval nucleus is lying transversely to the long axis of the trypanosome, the measurement given will not represent the greatest length of the nucleus.

Breadth.—The long and slender average 1·7 microns in breadth, the intermediate 2·1, and the short and stumpy 2·9. This measurement was made across the broadest part in 100 of each of the three varieties of this trypanosome.

Shape.—The long and slender are very similar in appearance to *T. brucei* and *T. gambiense*, but the intermediate differ to some extent in that the posterior extremity seems often to be more elongated than in the other two species, and blunter (Plate 11, figs. 1, 2, and 3; Plate 12, figs. 6, 8, and 9). The short and stumpy are also characterised by blunter posterior extremities, often suggesting the appearance of a hippopotamus head (Plate 11, figs. 8 and 9). On comparing these plates with those of *T. brucei* and *T. gambiense* this difference will readily be seen.

Contents of Cell.—In well-stained films the protoplasm of many of the trypanosomes shows granules, especially in the anterior portion of the cell.

Table V.—Percentage of Posterior Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Date.	Expt. No.	Animal.	Percentage of short and stumpy forms.	Proportion to all forms per 1000.
1912.				
Mar. 1	233	Monkey	7	17
„ 4	233	„	10	25
„ 14	233	„	6	15
Feb. 23	234	„	21	52
Mar. 1	234	„	1	2
Feb. 29	14	Dog	7	17
„ 26	157	„	16	40
„ 29	243	„	41	102
„ 29	13	Guinea-pig	6	15
Mar. 18	13	„	8	20
„ 14	165	„	7	17
„ 18	165	„	36	90
Feb. 29	166	„	17	42
Mar. 18	239	„	36	90
Feb. 22	235	Rat	7	17
„ 23	235	„	22	55
„ 27	235	„	38	95
„ 28	235	„	41	102
„ 29	235	„	39	97
Mar. 1	235	„	51	127
„ 4	235	„	29	72
„ 5	235	„	30	75
„ 6	235	„	36	90
„ 7	235	„	30	75
„ 8	235	„	38	95
„ 11	235	„	39	97
„ 13	235	„	45	112
Feb. 27	236	„	24	60
„ 29	236	„	32	80
Mar. 1	236	„	46	115

Nucleus.—The nucleus is oval in the long and slender and intermediate, and round or oval in the short and stumpy. One peculiarity about the nucleus of this species is that it is frequently placed far back in the body of the organism. This peculiarity is found in the short and stumpy non-flagellar forms (Plate 11, figs. 4 to 11), and more rarely in the intermediate (Plate 11, figs. 12 and 13); never in the long and slender. The percentage among the short and stumpy forms is often large, as will be seen by Table V. As the percentage of these non-flagellar forms is 24·8 of the whole, it is easy to calculate the percentage of the posterior nuclear forms to the whole body of this trypanosome.

Micronucleus.—Small and round, and situated, on an average, 2 microns from the posterior extremity in the long and slender, 1·6 in the intermediate, and 1·4 in the short and stumpy.

Undulating Membrane.—This, as in *T. brucei* and *T. gambiense*, is well developed and thrown into bold folds and undulations.

Flagellum.—The flagellum in the long and slender averages 5·8 microns (maximum 11, minimum 2), and in the intermediate 3·3 microns (maximum 9, minimum 1). There is no free flagellum in the short and stumpy forms. In *T. brucei* the short and stumpy, non-flagellated forms mostly lie between 13 and 21 microns. This is not so in the trypanosome under consideration, as the following table shows:—

Table VI.—Number of Flagellated and Non-flagellated Forms found among 1220 Trypanosomes of the Human Trypanosome Disease of Nyasaland.

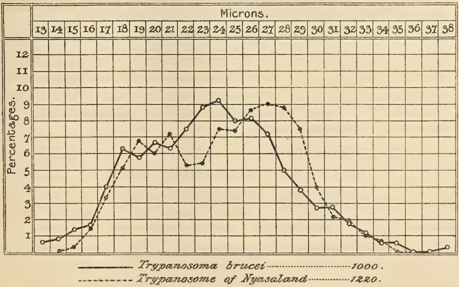
Length, microns.	Non-flagellated.	Flagellated.	Length, microns.	Non-flagellated.	Flagellated.	Length, microns.	Non-flagellated.	Flagellated.
Short and stumpy.			Intermediate.			Long and slender.		
14	1	—	22	5	60	25	—	91
15	4	—	23	2	64	26	—	107
16	19	—	24	—	93	27	—	110
17	42	—				28	—	104
18	62	1				29	—	87
19	74	7				30	—	49
20	56	19				31	—	27
21	37	54				32	—	23
						33	—	13
						34	—	7
						35	—	1
						36	—	1
	295	81		7	217		0	620

The above table shows that 81 of the intermediate forms are under 22 microns in length, and that seven short and stumpy forms are above 21 in length. If the trypanosomes are separated into non-flagellar and flagellated, then there are 24·8 per cent. of the former and 75·2 per cent. of the latter.

COMPARISON OF THE TRYPANOSOME OF THE HUMAN TRYPANOSOME DISEASE OF NYASALAND WITH T. BRUCEI.

The coloured plate which accompanies this paper, when compared with that of *T. brucei** or *T. gambiense*,† shows that the three species are much alike. The chief difference is the tendency of the Nyasaland trypanosome, as has already been pointed out, to show numerous posterior nuclear forms. The Commission has had no opportunity of examining *T. brucei* for posterior nuclear forms under the same conditions as the Nyasaland organism, but it may be assumed that *T. brucei* does not show these aberrant forms to the same extent, or the fact would have been noted long ago, no species having been studied so often or so closely as *T. brucei.* Another difference between the two species is the occurrence of the broad, blunt-ended forms—the hippo-headed—among the short and stumpy, a form which rarely, if ever, appears in Bruce's trypanosome.

CHART 2.—Chart giving Curves representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of *T. brucei*, and 1220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.



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* 'Roy. Soc. Proc.,' 1911, B, vol. 83, Plate 2.

† *Ibid.*, vol. 84, Plate 13.

But when we compare the curves representing the distribution by percentages in respect to length of 1000 and 1220 individuals of each species, the similarity of the two parasites is remarkable. In *T. brucei* there are 26 per cent. non-flagellated forms, in the trypanosome of Nyasaland 25 per cent.

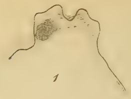
Again, if we divide the 1220 Nyasaland trypanosomes, by length, into short and stumpy (13–21 microns), intermediate (22–24 microns), and long and slender (25 microns and upwards), as has been done in the case of *T. brucei* and *T. gambiense*, the result is as follows:—

	Short and stumpy.	Intermediate.	Long and slender.
T. gambiense	51 ·2	23 ·1	25 ·7
T. brucei	32 ·8	25 ·5	41 ·7
Nyasaland trypanosome	30 ·8	18 ·4	50 ·8

This shows the percentage of the short and stumpy to be almost the same in *T. brucei* and the Nyasaland trypanosome, and the latter to be rather better off in the long and slender forms.

COMPARISON OF THE TRYPANOSOME OF THE HUMAN TRYPANOSOME DISEASE OF NYASALAND WITH T. RHODESIENSE (STEPHENS AND FANTHAM).

Dr. J. W. W. Stephens, of the Liverpool School of Tropical Medicine, at Sir David Bruce's request, kindly measured 1000 trypanosomes of the strain from a case of Sleeping Sickness from the Luangwa Valley, North-east Rhodesia, which he has named *T. rhodesiense.* This proposed new species is characterised by posterior nuclear forms, and a snout-like prolongation of the posterior extremity. These are also, as we have seen, characteristics of the Nyasaland trypanosome. The following chart has been prepared from the figures supplied by Dr. Stephens, and shows that the similarity of the curve prepared from 1000 individuals of *T. rhodesiense* to that prepared from 1220 individuals of the Nyasaland trypanosome is still more remarkable than in the case of *T. brucei.* Dr. Stephens measured 100 trypanosomes from man, 40 from the monkey, 40 from the horse, 40 from the dog, 40 from the rabbit, 100 from the guinea-pig, 600 from the rat, and 40 from the mouse.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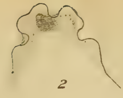
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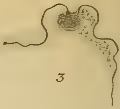
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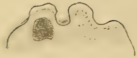
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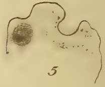
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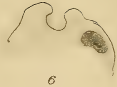
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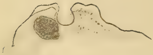
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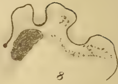
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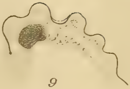
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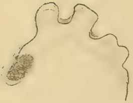
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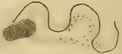
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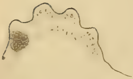
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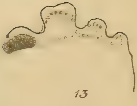
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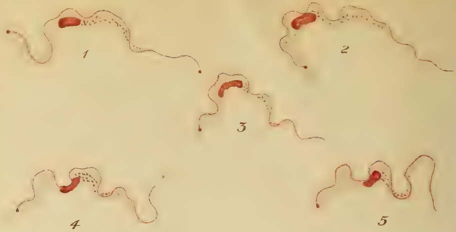
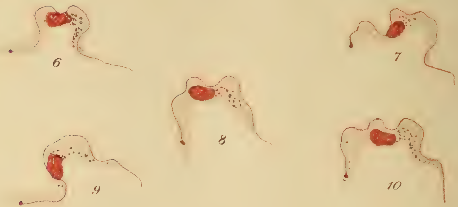
M. E. Bruce, del.



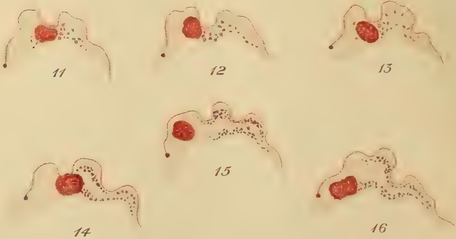
1 2 3 4 5

Long & Slender



6 7 8 9 10

Intermediate

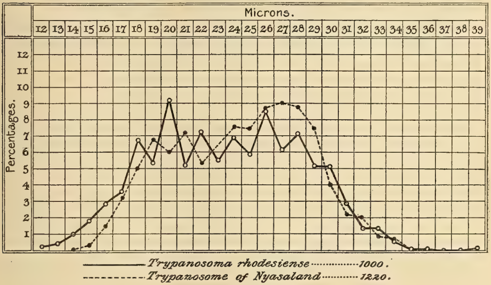


11 12 13 14 15 16

Short & Stumpy

M. E. Bruce, del.

Chart 3.—Chart giving Curves representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of *T. rhodesiense*, and 1220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.



Conclusions.

1. The trypanosome of the human trypanosome disease of Nyasaland is *T. rhodesiense* (Stephens and Fantham).

2. This is a distinct species, nearly related to *T. brucei* and *T. gambiense*, but more closely resembling the former than the latter.

3. From this it follows that the human trypanosome disease of North-east Rhodesia and Nyasaland is not the disease known as Sleeping Sickness in Uganda and the West Coast of Africa.

4. The native name "Kaodzera" might be used for this new disease to distinguish it from the older known Sleeping Sickness.

DESCRIPTION OF PLATES.

Plate 11.

Trypanosoma rhodesiense, posterior nuclear and blunt, hippo-headed forms found commonly in this species. × 2000.

Figs. 1, 2, and 3. Intermediate forms with blunt posterior extremities.

Figs. 4, 5, 7, 8, 9, 10, and 11. Posterior nuclear forms.

Fig. 6. Nucleus near anterior extremity.

Figs. 12 and 13. Posterior nuclear intermediate forms.

Plate 12.

Trypanosoma rhodesiense, Nyasaland, 1912, stained Giemsa. × 2000.

Figs. 1—5. Long and slender forms.

Figs. 6—10. Intermediate forms.

Figs. 11—16. Short and stumpy forms.

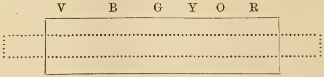
Negative After-Images and Successive Contrast with Pure Spectral Colours.

By A. W. Porter, B.Sc., F.R.S., Fellow of University of London, University College, and F. W. Edridge-Green, M.D., F.R.C.S., Beit Memorial Research Fellow.

(Received May 28,—Read June 27, 1912.)

The object of the research was to ascertain the appearance of pure spectral colours after the eye had been fatigued by light of a known wavelength or situated in a portion of the spectrum between two known wavelengths.

The method adopted was as follows:—In a dark room, in which, however, there was a certain amount of stray light, a horizontal spectrum as pure as possible was projected upon a screen. A portion of the retina of one eye was then fatigued by rigidly gazing at a portion of another horizontal spectrum which was isolated in the Edridge-Green colour-perception spectrometer.* The eyes were kept in a vertical position, that is, one over the other, so that the long axis of the after-image would be at right angles to the individual colours of the spectrum when the eye resumed its normal position. After the fatiguing light had been viewed for a period of about 20 seconds the eye was turned to the screen so that the after-image formed a band running right across the spectrum on the screen and occupying its centre. By this means any change either in colour or luminosity in the portion occupied by the after-image could be readily detected on account of the comparison with the colours of the unaltered spectrum seen above and below (see Diagram).



The dotted line shows the projected after-image on the screen spectrum. The fatiguing light was much stronger than the reacting light.

1. The eye was first fatigued with pure red light occupying the region λ 654–λ 675. When the eye was turned to the spectrum on the screen the following changes were noted in the region occupied by the after-image: the extreme red was slightly diminished; there was no perceptible action on the

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* 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 458.

orange, yellow, or green. There was strong action on the blue and violet, these became much darker and bluer along the line of the after-image.

2. The fatiguing light was λ 654–λ 675 as before, but a filter was interposed in the path of the rays forming the screen spectrum so that only red and orange appeared upon the screen. After the fatigue the red disappeared but the orange remained. This was tried with a screen spectrum fairly strong and also very weak, with the same result.

3. After exposure to λ 654–λ 675 (orange-red) a sodium flame was viewed and found to be very little affected in the region of the after-image (no effect, A. W. P.) (slightly greener, E.-G.), though the green-blue after-image was very strongly marked on either side of the sodium flame.

4. The fatiguing range was λ 619–λ 631 (orange). The dark blue after-image was seen right across the spectrum except in the region of the orange, which appeared unaffected.

5. The fatiguing range of λ 619–λ 631 was also used with a screen spectrum showing only orange and red. The same result was obtained as with the red, namely, the red disappeared, being replaced by the green-blue after-image, whilst the orange appeared unaffected.

6. Fatiguing range, λ 585–λ 595 (orange-yellow). Purple after-image seen right across spectrum. The red was affected most. The general impression was that the band appeared as if painted over the spectrum.

7. Fatiguing range, λ 545–λ 550 (yellow-green). Purple after-image similar to above and effects similar to λ 585–λ 595, least effect in orange.

8. Fatiguing light, λ 496–λ 500 (blue-green). Exactly the same effect as before, with λ 545–λ 550. Orange least affected; purple not red after-image. When an after-image was projected on a sodium flame, no effect (A. W. P.), (slightly redder, E.-G.).

9. Fatiguing light, λ 475–λ 480 (blue). After-image reddish-purple, no action on red and orange, the after-image seen over the rest of the spectrum.

10. Fatiguing light, λ 445–λ 455 (blue). After-image yellow-green (A. W. P.) or orange (E.-G.); violet and blue cut off, green and red made yellower. When a filter was used so that only green and red were seen on the screen, the green was cut out by the after-image, but the red was unaffected.

11. Fatiguing light, λ 425–λ 436 (violet). The after-image was green, which made the violet and blue appear green, and made the green appear slightly a yellower green, and did little or nothing to red or orange.

12. Another method was now adopted, so that a definite and sudden reduction of luminosity in the spectrum on the screen could be obtained when required. This was accomplished by the interposition of Nicol prisms in the path of the light forming the spectrum on the screen. The eyes

being vertically over one another, the horizontal spectrum on the screen was viewed through the Wratten and Wainwright red filter, so that only red and orange were visible. The spectrum being at its greatest luminosity was viewed for about 20 seconds, and then, at the same time as the filter was removed and the eye was turned to its normal position, the Nicol was rotated and the intensity of the spectrum much reduced. The blue band under these conditions stretched right across the spectrum, whereas when the spectrum was not reduced so much (though still much reduced) the orange and yellow were predominant, and showed no change.

13. The same process was tried by fatiguing the eye with light through a Wratten and Wainwright blue filter (blue-green). There was no effect unless the spectrum on the screen was very much enfeebled; in that case a practically black after-image, but if a stronger fatiguing source were used a feeble red after-image, was seen.

14. A Wratten and Wainwright green filter was now used in the same way, and the screen spectrum was afterwards much reduced. A purple band, having least effect on the brightest parts, yellow and orange, was seen stretching across the spectrum.

15. A yellow band was formed on screen of about λ 570, obtained by use of a second slit placed in the focal plane where the spectrum was first formed; the light let through being then projected on screen.

It was not quite pure, but contained a little yellow-green and orange mixed with the yellow; it, however, appeared as pure yellow on the screen. When the eye was fatigued by light through the red filter, the after-image on the yellow band was grey or black; no tinge of colour was seen unless fatiguing light was very bright (direct arc seen through red filter), when the yellow image on screen became green.

The eye was now fatigued by light from a separate spectroscope, and the effects noted on the subsequent appearance of the after-image after the spectrum on the screen had been viewed. The process adopted was as follows:—

The eye was fatigued by light of a definite wave-length for 20 seconds and the appearance of the after-image on the spectrum on the screen noted. The eye was then moved so that the after-image was visible on a dark part of the screen.

16. Fatiguing light of the region of the D lines was first selected. After the eye had been fatigued with this, on looking at the spectrum on the screen a blue band appeared except on the brightest region (green, yellow, and orange). On then looking at another portion of the screen the blue band appeared continuous, *i.e.* without any change of tint or intensity, while the after-image of the screen spectrum appeared above and below it.

17. When the same was done after fatiguing with blue light the green of the spectrum also changed, becoming a yellower green, and the image seen afterwards on a fresh part of the screen was an orange band with the after-image of the spectrum above and below it.

18. On fatiguing with red light λ 640 the blue-green after-image was seen over all the spectrum with the exception of the yellow, orange, and brightest red; the after-image on a fresh part of the screen appeared as a blue-green band which was cut into two by the after-image of the spectrum corresponding to the yellow region. When this experiment was repeated, the spectrum being viewed for a little longer than before, it was found that the blue-green band was cut through by the whole of the spectrum after-image, but it was noticed that the after-image of the spectrum faded away the faster of the two, and then the blue-green band ran continuously across.

19. The eye was then fatigued by red on the screen, using the red filter; the eye was then removed to the eyepiece of the spectroscope, the green-blue image ran across superimposed upon the image whatever part of the spectrum was selected.

20. The eye was then fatigued by different regions in the Edridge-Green spectrometer. A monochromatic region was selected and the eye fatigued in a vertical position (*i.e.* one eye over the other) for 20 seconds. On assuming the normal position the after-image was seen to cross the region.

No matter what portion of the spectrum was selected the after-image where it crossed the spectral band was seen as a grey square.

Conclusions.

The first point which was evident was the very great importance of the intensity of the light which was used, especially in relation to the reacting light. In this respect the results are different from those in which the whole eye is fatigued by a very intense light, as in the experiments of Burch,* or by comparatively weak light acting for a prolonged period upon the whole eye, as in the experiments of Edridge-Green and Devereux Marshall.† As the eye was kept rigidly fixed during the fatiguing process, a very clear cut negative after-image was produced which, when thrown on the screen spectrum, enabled close comparison to be made with adjacent parts. The stability of the after-image was remarkable; it did not change colour, and was not influenced by subsequent light falling on the retina when this was not of too great intensity. The after-image was in every case darker than any dark object on which it was projected. If the portion of brain having the function of the perception of colour be continually receiving impulses which, affecting it and the visual centre, cause the sensation of light (Eigenlicht) which is seen in the absence of all light stimulation, and the whole retino-cerebral apparatus be fatigued by light of a certain wave-length, a negative after-image will appear through simultaneous contrast. If one portion of the visual area be less sensitive for impulses caused by light of a certain wave-length (for instance, red), and the adjoining areas be stimulated by impulses corresponding to light of all wave-lengths, the image corresponding to the fatigued area will be relatively blue-green to the images corresponding to surrounding areas. This explanation on the Edridge-Green theory of colour-vision is in accordance with the other facts of simultaneous contrast.* It would appear as if an inhibitory process took place which prevented light, unless it reached a certain intensity, from being perceived. In the present state of our knowledge it is impossible to say at what point in the nervous track the inhibitory process takes place, whether there be obstruction to the impulse at any point of the nervous track, or whether the effect be chiefly central. It is to be noted that when an effect is produced there is very little change in most cases, except when the intensity of the reacting light is the same or less than that of the fatiguing light.

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* 'Phil. Trans.,' 1899, B, p. 1.

† 'Trans. Ophth. Soc.,' 1909, p. 211.

The negative after-image is much darker, more difficult to produce, and more evanescent in the absence of all external light as when black velvet and the hands are placed over the eyes. It is obvious, therefore, that external light has an influence on negative after-images. It is impossible to explain these facts on the Hering and Young-Helmholtz theories of colour-vision :—

I. Hering.—The complementary to the exciting light is never strengthened in the spectrum on the screen by the after-image, and the after-image is not surrounded by the primary colour as it should be according to this theory.

II. Young-Helmholtz—

1. The effect of fatiguing the eye with a monochromatic region produces a uniform grey band across this region. On the Young-Helmholtz theory this should vary in colour and luminosity across its breadth.

2. There is no alteration of the after-image by observation of the second spectrum.

3. On the Young-Helmholtz theory the after-image should change colour on fading, because of the varying amount of fatigue of the hypothetical colour sensations. This is not the case.

4. Regions like violet, after fatigue to red, should be very little affected, but they are the most affected.

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* 'Roy. Soc. Proc.,' 1912, B. vol. 84, p. 546.

5. The fatiguing light should chiefly affect the region used for the fatigue. This is not the case.

6. An after-image should not be seen in the absence of all external light, except such as would arise from the Eigenlicht if this subsidiary hypothesis of Helmholtz be allowed. In the latter case the after-image should change colour as in all other cases according to this theory.

7. On the Young-Helmholtz theory yellow should change to green after fatigue to red, or to red after fatigue to green. This is not the case.

The Relation between Capillary Pressure and Secretion. II.—*The Secretion of the Aqueous and the Intraocular Pressure.*

By Leonard Hill, F.R.S., and Martin Flack (Eliza Ann Alston Research Scholar).

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(From the Physiological Laboratory, London Hospital Medical College, London Hospital Research Fund.)

Many observations have been recorded of the aqueous or intraocular pressure. The method generally used has been to pass a hollow needle through the cornea and connect this with a manometer. J. Herbert Parsons* says: "The first essential is . . . that no fluid shall enter or leave the eye whilst the needle is being inserted." It is no less essential that the aqueous shall not escape along the track of the needle or into the manometer. Although expressing the opinion cited above, Parsons gives the figure of a needle employed by him, which not only has an open end, but also two side holes, one nearer to, one farther from, the open end; so contrived, he says, to give free admission to the aqueous fluid. Such a needle seems to us especially liable to allow the escape of the aqueous during insertion. Starling and E. E. Henderson† used a needle with an open eyehole, from which fluid was allowed to escape at a pressure conjectured to be greater than the pressure of the aqueous. By this means they believed to prevent the escape of aqueous during insertion. It is not certain that this means is effectual, since pressure on the eyeball raises the intraocular pressure greatly, and considerable pressure may be required at the moment of insertion. The method also permits the entry of fluid into the eye should the pressure of the fluid employed be greater than that of the aqueous.

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* 'The Pathology of the Eye,' vol. 3, p. 1049. Hodder and Stoughton, London (undated).

† 'Journ. Physiol.,' 1904, vol. 31, p. 305.

We have measured the aqueous pressure by means of a specially constructed hollow needle, used in conjunction with the method employed by one of us (L. H.) when measuring the intracranial or cerebro-spinal fluid pressure.* The needle (fig. 1) consists of two hollow tubes, one revolving within the other.

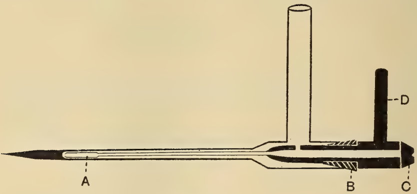

Fig. 1.

One end of the outer tube has a solid needle point attached. Each tube has a side eyehole, which in one position correspond one with the other. On revolving the inner tube through a quarter of a circle the holes no longer correspond and are closed. An index arm attached to the inner tube shows when the needle is closed and when open. This L.H. needle—as we shall call it—is connected by rubber tubing to a short length of capillary glass tubing, which in turn is connected by a T-piece with a manometer and with a pressure bottle. The pressure bottle is balanced by a weight and can be raised or lowered with ease (fig. 2). The whole system is filled with Ringer's solution, and an air bubble introduced within the capillary glass tube to act as an index. We find it convenient to introduce this air bubble by means of a hypodermic syringe, the needle of which is inserted through the rubber tube at its junction with the capillary glass tube. The zero position of the air bubble is marked while the needle is held open at the level of the eye of the animal, the surface of the solution in the pressure bottle being at the same level. The needle is then closed and made to transfix the cornea; the inner tube is turned so as to open the needle, while, at the same time, the pressure bottle is raised until the air index is restored to the zero mark and the pressure of the aqueous compensated. The reading of the manometer gives the pressure in centimetres of water. The method of counterbalancing the pressure prevents the entry of aqueous into the manometer. The same method was employed by v. Schulten and by E. E. Henderson and Starling. These last two researchers, however, introduced the air bubble index after the needle had been inserted, and then found the pressure which kept it stationary. Taking this into consideration, also the fact that they inserted the needle with fluid flowing from it at a pressure conjectured to be greater than the aqueous pressure, we conclude that their measurements do not give with certainty the normal intraocular pressure.

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* Leonard Hill, 'The Cerebral Circulation.' J. and A. Churchill, London, 1896.

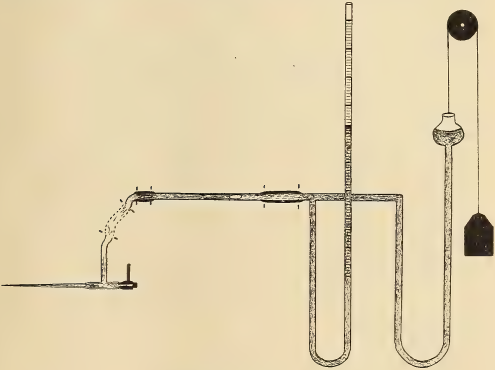

Fig. 2.

In some preliminary experiments carried out by Thomson Henderson and one of us (L. H.), an open needle was used and readings were obtained which seemed to show that the intraocular pressure approximated to the intracranial pressure; the two varied in the same way concomitantly with changes in the general circulatory pressure. It seemed not unlikely that these readings were vitiated by the escape of fluid during insertion, or afterwards, along the track of the needle, and were thus too low. The fluid may escape when the open eyehole of the needle is half inside and half outside the cornea. Also, when the small cylindrical needle has been passed through, it is difficult to prevent the leakage of fluid along its track. Any loss of fluid lowers the pressure and lessens the tension of the eyeball considerably. Thomson Henderson recognised the need for the invention of a proper needle, but opportunity did not allow him to continue this research. The L.H. needle is cone-shaped and somewhat larger than a hypodermic needle; the cornea grips it tightly, and since it is closed no fluid escapes at the moment of insertion. With practice, we have come to introduce the needle, as a rule, without leak. In our earliest experiments we often failed to do so and obtained readings which were too low. A possible source of error in the method arises from the volume of the needle introduced within the eyeball. If blood and not aqueous fluid is displaced from the eyeball in an amount corresponding to the bulk of the needle introduced, the intraocular pressure may rise and the readings be too high. The needle might act as does a clot, or a foreign body, when introduced into the intracranial cavity; by taking up room a clot expresses blood from the cerebral veins and increases the pressure in the exsanguined part from capillary-venous towards arterial pressure. If the bulk be small compared to the whole the result will be negligible. We have tested whether this be so by comparing the readings taken with the L.H. needle inserted in one eye and a hypodermic needle inserted in the other, the latter reading being taken immediately after insertion of the needle, before leakage has taken place. When introduced with a sudden thrust we obtain, with a small hypodermic needle, readings quite as high as with the L.H. needle. In some cases the two needles were inserted into the same eye. Since these readings (Table I) closely corresponded we have no reason to doubt the accuracy of our method.

Table I.

Animal.	Anæsthetic.	L.H. needle.	Hypodermic needle.	Remarks.
		mm. Hg.	mm. Hg.	
Cat	Ether	54	52	B.P. 100. Readings taken in different eyes. Hypodermic quickly fell to 18 mm. Hg.
Dog	Morphine Ether	53	46	Both readings in same eye. When hypodermic read 46, L.H. also read 46 mm. Hg.
Cat	Ether	51	49·8	Needles in opposite eyes. Hypodermic gradually sank to 30 mm. Hg.
Cat	Ether	50	50·5	Needles in opposite eyes.
Kitten	,,	36	36	,, ,,
Dog	Dead	16	15	Right eye. } Needles in same eye.
		15	15	Left ,, }

The average intraocular pressure as measured by 15 or more observers is 20–30 mm. Hg. In several of our animals, especially those anæsthetised with ether, we have obtained readings much exceeding these figures—45–65 mm. Hg (Tables I and II). With chloroform as the anæsthetic, especially in deep anæsthesia with low blood pressures, we have obtained in some cases readings approximating more closely to those of other observers. This has also been the case when any leakage of aqueous has occurred. After such leakage the intraocular pressure may measure 20–30 mm. Hg, and the eye then feels to the finger much less tense than does the other normal eye.

Table II.

Anæsthetic.	Normal intraocular pressure with L.H. needle.		On squeezing the belly. Rise.	On stopping the heart beat. Fall.	Remarks.
	cm. H_2O.	mm. Hg.	cm. H_2O.	cm. H_2O.	
Chloroform ...	31	23	—	—	
,, ...	28	21	$12\frac{1}{2}$	12	
A.C.E.	73	54·4	—	—	80 cm. H_2O on very light anæsthesia.
Urethane	38	28·3	—	—	
A.C.E.	73	54·4	—	—	Reading 70 cm. H_2O after 1 hour.
Ether............	76	56·7	12	22	B.P. 118 mm. Hg.
,, ...	60	45	12	20	B.P. 110 mm. Hg.
,, ...	87	65	13	60	B.P. 190 mm. Hg. On pithing medulla, B.P. fell quickly to 44 mm. Hg, eye to 54 cm. H_2O = 40 mm. Hg.
,, ...	84	62·7	—	—	
,, ...	68	50·7	—	—	
Chloroform ...	22	16·4	—	—	Animal deeply under, leak.
,, ...	43	32	—	21	By anæsthetising deeply went to 29 cm. H_2O, on light anæsthesia rose to 66 cm. H_2O.
,, ...	36	27	—	—	On deep anæsthesia fell to 26 cm. H_2O.
Ether............	55	41	—	—	
,, ...	68	50·7	10	26	
,, ...	72	53·7	6	13	
,, ...	84	62·7	8	—	
,, ...	66	49·2	12	—	

Since the intraocular pressure is raised by traction or by pressure on the eyeball, it is necessary to support the needle. We always hold the needle in such a way as to give the lowest readings of pressure. When water is forced into the anterior chamber of an excised eye at a pressure of about 50 mm. Hg, the tension of the eye feels about the same as that of the human eye. We are certain, therefore, that the normal intraocular pressure is much higher than has been supposed.

In some of our experiments we have measured the intracranial pressure, or that of the cerebro-spinal fluid, at the same time as the intraocular pressure. We find that we can pass another needle of similar construction through the occipito-atlantal membrane and thus compare under the same conditions the aqueous and the cerebro-spinal fluid pressure. In this part of the research we used cats and a few dogs. The animal is anæsthetised with ether, and after a tracheal cannula has been inserted, it is placed belly downwards on a flat, hot-water tin with its head over the end of the tin. The occipito-atlantal membrane is exposed by separating the muscles from the occipital bone after making a median longitudinal incision; weighted hooks are used, two to keep open the sides of the incision, one, fastened to the anterior end of the wound and passed over the snout, to keep the head in position. The needle is passed through the membrane from side to side so that its eyehole opens into the bath of cerebro-spinal fluid. The pressure of the aqueous is much higher than that of the cerebro-spinal fluid, or intracranial pressure, *e.g.* one may be 5–10 cm. of water, the other 30–60 cm. The air indices in either case pulse with each heart beat, but while the index of the cerebral instrument follows with large excursions the respiratory variations of the venous pressure, that of the eye remains unaffected. The pressure of the aqueous is so high that the variations of venous pressure do not influence it.

Starling and E. E. Henderson also noted the fact that the intraocular pressure is not affected by changes in the general venous pressure.

The observations of others (v. Schulten, Starling and Henderson, etc.) show that the intraocular pressure varies in the same direction as the arterial pressure. In this respect the aqueous and the cerebro-spinal pressures go together. Ligation of the carotid artery on the same side lowers the aqueous pressure 5–10 mm. Hg; compression of the abdominal aorta raises it 10–20 mm. Hg; section of the spinal cord in the cervical region lowers it 10–15 mm. Hg; stoppage of the heart lowers it 10–12 mm. Hg.

In general we confirm the above results. Thus, tightening a string round the neck caused a fall of 15 mm. Hg in the intraocular pressure. During stimulation of the depressor nerve the arterial pressure fell 36 mm. Hg, the intraocular 8 mm. Hg. Stimulation of the cervical sympathetic caused a rise of intraocular pressure of 1–2 mm. Hg, followed by a fall of 4–5 mm. Hg. Starling and E. E. Henderson ascribe the rise to contraction of the orbital muscle and the fall to vaso-constriction of the intraocular arteries. Dr. W. T. Lister examined the eye of one of our cats with the ophthalmoscope and was unable to note any constriction of the retinal vessels during stimulation which produced full dilatation of the pupil. We would point out that dilatation of the pupil must cause expulsion of the blood in the iris, and that the volume of blood so expelled must be made good either by a secretion of aqueous or by expansion of other blood-vessels, *e.g.* in the ciliary region. The latter probably takes place, and as the dilating iris shrinks the veins which drain it dilate in the ciliary region. We have no evidence to offer as to how the small changes of intraocular pressure occur during the stimulation of the cervical sympathetic. During asphyxia we obtained a rise of 12 mm. Hg in the eye and 20 mm. Hg in the brain. The intracranial pressure is much lower than the intraocular and is affected by any rise of the general venous pressure, while the intraocular is not. Therefore, the intracranial pressure is raised during asphyxia both by the rise in arterial and in the general venous pressure. On squeezing the heart the intraocular pressure falls 15–20 mm. Hg, while the intracranial rises 5–7 mm. Hg. Here again the difference is due to the greater effect of the venous rise on the brain. Compressing the abdomen causes a rise of intraocular pressure (3–10 mm. Hg.) and of intracranial pressure (5–15 mm. Hg). Here also the rise in venous pressure adds itself to the arterial in the case of the brain and not in the case of the eye.

If the heart be divided, there occurs a rapid initial fall of pressure, followed by a slow fall. A well-recognised sign of death is the flaccid cornea. The initial fall is due to the fall of arterial pressure; the residual pressure demonstrates the existence of an intraocular pressure which is not circulatory but secretory in origin, a point of importance. The tense form of the eyeball, and the perfection of the eye as an optical instrument, depend primarily on the secretory activity of the ciliary processes. It would be much to the disadvantage of an animal if the eye became flaccid at once on stopping the arterial blood supply to the eye. The intraocular pressure is of circulatory origin, and varies with the arterial pressure, but it is the secretory power of the cells of the ciliary processes which determines the circulatory pressure in the eyeball.

Turning to the excellent historical survey given by J. Herbert Parsons* of past work on this subject, we cannot find therein any clear conception of the physical conditions which underlie the intraocular pressure and the circulation in the eyeball. Leber, Parsons, Henderson, and Starling conceive that the capillary pressure is much higher than the intraocular, *e.g.* in the ciliary processes, and that the aqueous is a filtration product depending on the difference of pressure on either side of the membrane. "What is of prime importance," writes Parsons,† "is the fact that everything tends to show that the secretion of aqueous is directly dependent upon the

* *Loc. cit.*, vol. 3, pp. 1040—1070.

† *Loc. cit.*, vol. 3, p. 1048.

intracapillary pressure, and that it varies *pari passu* with that pressure." Again, on pp. 1046–7: "The capillary pressure in most parts of the body is normally about 15 to 20 mm. Hg (Starling). No means has yet been devised for measuring the intraocular capillary pressure, since the veins are so small, and anastomose so quickly and profusely after leaving the eye, that it is impossible to measure the venous pressure." . . . "In any case," he continues, "it is clear that, with an external pressure—the normal intraocular pressure—of 20 to 30 mm. Hg, the capillary pressure must be considerably above this level, and we shall probably not be far wrong in considering it equal to 40 or 50 mm. Hg."

We maintain, on the other hand, that the physical conditions which pertain in the eyeball are closely similar to those which pertain in the cranio-vertebral cavity, and that the results obtained and the conclusions drawn by one of us* in regard to the cerebro-spinal fluid and the cerebral circulation hold good for the eye. Thomson Henderson† alone has upheld this point of view, and has expressed it with great clearness. "The eyeball is contained by an unyielding capsule of fixed volume, and the circulation is conducted through an elastic system of vessels. The intraocular pressure is the lowest circulatory pressure in the closed sphere of the eyeball." In the case of the intracranial contents, the pressure of the cerebro-spinal fluid, the pressure in the cerebral veins, and the pressure required to overcome that of the brain against the skull wall, and flatten it when it bulges into a trephine hole, are found to be one and the same.

The following passage shows that Parsons‡ rejects the application of these findings to the eye: "We know that the outflow of blood from the eye by the veins is usually a steady stream. It therefore follows that the venous pressure must also be considerably above the average in other parts of the body, since, if it were below the intraocular pressure, the veins would collapse, and would only be reopened when the internal pressure rose above that level. It has been thought that increase of intraocular pressure would tend to close the venæ vorticosæ, but experimental investigation does not bear out this view. The very oblique course of these vessels through the sclerotic is further a protective mechanism against kinking, such as might be produced by the action of the ciliary muscle in dragging forward the equatorial region of the choroid. As long as the intraocular tension is constant, the fluid contents of the globe must be constant, any outflow of fluid, either blood or lymph, being equalised by a corresponding inflow of

* Leonard Hill, *loc. cit.*

† Thomson Henderson, 'Glaucoma.' Arnold, London, 1910.

‡ *Loc. cit.*, vol. 3, p. 1047.

secretion. Under these circumstances, the eye is temporarily a rigid box, and, as is shown to be the case in the brain by Leonard Hill, the venous pressure is equal to the intracranial pressure, so here, too, the venous pressure will also be equal to the intraocular pressure. This will not hold for variations of intraocular pressure, as it does in the case of the brain, because under these circumstances the eyeball is no longer a rigid case." Yet Parsons* cites Koster's experiments, which prove that an increase of intraocular pressure from 19 mm. Hg to 70 mm. Hg only increases the radius of the globe 0·027 mm., or the volume 45 cu. mm., or 7/1000 of the original volume. (Koster's experiments were done on the freshly excised eye.)

E. E. Henderson and Starling† sought to find evidence to prove that the aqueous is a filtration produced by a higher capillary pressure in the ciliary processes. They opened the anterior chamber and measured the outflow of aqueous, and observed the effect of obstructing the aorta, and so raising the arterial pressure in the eye. These conditions are wholly abnormal. Great dilatation of the vessels of the ciliary processes and the iris results, because, as we hold, the capillaries are no longer supported by the exactly equivalent counter-pressure of the aqueous. The protein content of the aqueous rapidly rises from the normal, less than 0·05, to 3, 4, and even 5 per cent., the fluid becoming coagulable. If the drop in intraocular pressure is suddenly made, red corpuscles may appear in the fluid on raising the arterial pressure, the epithelium of the ciliary processes changes, and blisters may form beneath it (Greeff). The operation actually converts the normal conditions, which are quite otherwise, into those supposed to exist by E. E. Henderson and Starling, and the result is to give a wholly different fluid. To open the eyeball has the same result as abrading the skin. The plasma escapes through the damaged and unsupported capillaries. After we have freely opened and emptied the anterior chamber, we have observed the effect of raising the arterial pressure by compressing the abdomen. The iris and the ciliary body are congested, and with the lens bulge forwards, coming into contact with the cornea. This is because the pressure of the aqueous no longer balances the capillary venous pressure. Compressing the abdomen after opening the anterior chamber causes blood to escape from the iris. A convulsive movement of the animal may have the same effect, hence the bleeding which occurs in lesions of the eyeball. Plasma escapes from the anterior chamber under these conditions, just as it does from the skin when abraded. Starling and E. E. Henderson felt the unconvincing nature of the results so obtained, and turned to another method of enquiry. After introducing a hollow needle, and balancing the pressure, they divided the heart of the animal, and measured the rate of movement inwards of the air index; they say: "This rate must be equal to the rate of production of fluid previously obtaining in the eye under normal conditions of circulation." Parsons says:* "That constant reabsorption of the fluid secreted is taking place is shown by the injection of an indifferent fluid into the anterior chamber of a freshly removed eye under constant pressure." . . . "The fluid can be seen to exude from the cut ends of the anterior ciliary and vortex veins, and the pericorneal region becomes injected with the coloured fluid (Priestley Smith)." Again, "The difference between the amounts of fluid entering the living and the dead eye will give the amount of fluid secreted by the eye at the given pressure (Adamük, Jesner, Niesmanoff)."†

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* *Loc. cit.*, vol. 3, p. 1042.

† *Loc. cit.*

In our opinion these are amazing claims to make, and show how far the authors are from what we hold to be a true conception of the conditions in the living eyeball. As in the case of the brain, the mean capillary-venous pressure and the intraocular pressure are one and the same. Each pulse keeps up the pressure, brings into play the elasticity of the eye and maintains the onflow and outflow of blood. Evidence shows that the aqueous is secreted by the ciliary processes and absorbed by the capillary-venous network of the iris and the iridial angle. Nuel and Benoit‡ have traced the paths of absorption by injecting Chinese ink into the living eye. There is nothing in the structure of the iris, ciliary processes, or retina which can act as a rigid membrane and allow an inequality of pressure between the blood in the capillaries and veins and the fluid in the aqueous or vitreous outside. Measurement has shown that the vitreous and aqueous pressures are the same (Bellarminoff, Hamburger, Priestley Smith). The whole eyeball must be at one and the same fluid pressure, namely, the lowest hydrostatic pressure, and this is the pressure in the veins at their exit through the sclerotic. In the case of the brain we know there is no difference of pressure on either side of the capillary membrane; measurement has proved this, the capillary-venous pressure and cerebro-spinal fluid pressure are the same. When, as Starling and E. E. Henderson did, we divide the heart of the animal, the air index moves towards the needle in the anterior chamber, first because the blood at once leaves the eyeball, secondly, because the veins are no longer filled and there is within them no pressure to counter that of the aqueous outside. The spaces of Fontana are covered only by a layer of endothelium less than 1μ thick ;* by this, supported as it is by an equality of fluid pressure, aqueous on one side, venous blood on the other, the normal absorption of the aqueous is controlled. In the bloodless eye of the dead animal the spaces are converted into filtering structures by forcing fluid into the anterior chamber. Unsupported on one side, the delicate membranes are ruptured and the fluid drains away. Since the pores of this filter are small, serum will naturally escape more slowly than salt solution. Starling and E. E. Henderson found this to be the case. No further refutation is needed of their conclusion that by their second method the normal rate of secretion can be measured.

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* *Loc. cit.*, vol. 3, p. 966.

† Parsons, *loc. cit.*, vol. 3, p. 967.

‡ 'Arch. f. Ophthal.,' 1895, vol. 41.

The only convincing method of studying the normal secretion of the aqueous is that introduced by Ehrlich,† who traced it in rabbits by a subcutaneous injection of fluorescein. He states that the dye can be observed flowing into the anterior chamber from behind and round the edge of the iris, producing a characteristic median vertical line which he regards as the resultant of fluid eddies. In the young animal the fluorescent line may appear within five to seven minutes after the subcutaneous injection has been made. In the old the phenomenon may fail or appear after a period twice or thrice as long. The method shows that there is a current of aqueous entering the anterior chamber, therefore the aqueous must circulate through the chamber, and this more actively in the young. After the cornea has been punctured and the aqueous let out of the eye, the dye enters rapidly, so that within five to six minutes after the injection the anterior chamber appears full of fluorescent green water. While as little as one part of fluorescein in 2,000,000 is visible against a dark background, the concentration of the dye in the blood at any one time must be very low. Ehrlich draws the conclusion that the rapidity with which the dye appears in the aqueous shows that a process of active secretion is taking place, which concentrates the dye. Further work has shown that, after intravenous injection, the dye may appear in the aqueous within a minute, the ciliary processes becoming rapidly stained. After a subcutaneous injection the colour of the aqueous is most intense in three to four hours, fading away in six to 24 hours. Since fluorescein injected into the vitreous remains for two or three weeks there can be no rapid circulation of fluid in the vitreous chamber. Subconjunctival injection of suprarenal extract not only prevents the appearance of Ehrlich's line, but if the anterior chamber be punctured, it refills very slowly and with a fluid containing little if any excess of protein (Wesseley). After the application of eserine or pilocarpine to the eye, the fluorescein appears and disappears more quickly, a result attributed to the increased surface of the iris.

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* Thomson Henderson.

† Ehrlich, 'Deutsch. Med. Wochen.,' 1882, Nos. 2–4.

To Ehrlich has been attributed the suggestion that an appreciable amount of intraocular fluid may be secreted directly into the anterior chamber by the anterior surface of the iris (Starling and Henderson, also Parsons). We find nothing to confirm this in Ehrlich's paper. He describes the fluorescein as coming round the edge of the iris, the context clearly showing that he conceives the secretion as taking place from the ciliary processes. "Aus diesem leicht auszuführenden und geradezu nicht verfehlenden Versuche ergiebt sich ohne Weiteres, dass bei der Regeneration des Kammerwassers zunächst die Vorderfläche der Iris vollkommen unbetheiligt ist, und dass diese ausschliesslich von der hinteren Kammer aus erfolgt."

That there can be no difference of fluid pressure inside the eyeball, and therefore, that the aqueous is a true secretion, we have shown by a series of experiments in which the flow of blood is observed from the venæ vorticosæ during alterations in the pressure of the fluid in the anterior chamber. The rim of the orbital cavity is carefully cut away and the exit of one of the vortex veins exposed. Our needle is then carefully introduced into the anterior chamber and the pressure measured. A snick is now made in the vortex vein so that the blood flows freely out. Upon increasing the pressure in the eyeball by raising the pressure bottle the flow of blood becomes less and less until finally it ceases to flow.*

Measurement of the pressure at which this cessation of the flow occurs shows that it corresponds closely with the carotid pressure of the same side. Thus, to give an illustrative example, it was found that it required 160 cm. of water (120 mm. Hg) to stop the flow of blood from an eye the normal aqueous pressure of which was 69 cm. of water (51·5 mm. Hg). The carotid pressure of the same side taken immediately afterwards was 118 mm. Hg.

V. Schulten† carried out a somewhat similar experiment in order to measure the pressure in the ophthalmic artery. He inserted a cannula into the vitreous and drove in saline solution under increasing pressure until the blood flow in the retinal arteries was just stopped as observed ophthalmoscopically. It was found to be only a few (2–15 mm. Hg) below that in the large arteries.

In these experiments the fluid pressure is equally distributed throughout the eyeball, and the venous pressure rises *pari passu* with the fluid pressure, for the veins are reduced in volume and blood expressed until the whole vascular system of the eye becomes a rigid system, the arterial pressure pertaining alike to arteries, capillaries, and veins. The outflow of blood from the veins of the eye ceases when the fluid pressure is made greater than the arterial pressure. It is obvious in this case that the pressure of the fluid outside and the blood inside the capillaries must be one and the same. The conditions are similar to those which are established in the brain when an artery ruptures and blood escapes at arterial pressure into the cranial cavity. The veins are compressed and the blood within them expressed until the pressure in the exsanguined brain rises to arterial pressure. When the hæmorrhage ceases and the blood clots the pressure in the brain still remains high because of the volume of the clot, which takes up the room formerly occupied by blood in the cerebral vessels. The pressure which rules in the exsanguined part is the arterial pressure. The cause of the cerebral pressure is thus the arterial pressure, but the height of that pressure is determined not only by the force of the heart beat but also by the volume of fluid extruded within the skull cavity. The difference between the brain and the eye is that the secretory pressure within the eye is maintained at a much higher level.

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* The iris dilates when the pressure is raised, and this dilatation is prevented by section of the cervical sympathetic nerve.

† Parsons, *loc. cit.*, vol. 3, p. 1046.

The cerebro-spinal fluid is secreted at a pressure about the same as the general venous pressure, and saline injected into the intracranial cavity at a higher pressure is rapidly absorbed; the capillaries and veins afford the pathway for absorption. If saline be run into the subdural space at a high pressure the outflow from the cerebral veins ceases when the arterial pressure is exceeded by that of the fluid forced into the skull cavity. The same, we show, holds good for the eye.

In the case of the salivary gland, enclosed as it is in a capsule, the secretory pressure regulates the circulatory pressure. When the secretion is obstructed, the secretory pressure rises, the distension of the alveoli narrows the veins and the capillary venous pressure and the tenseness of the gland rise *pari passu* with the secretory pressure; the arteries are dilated, the whole circulatory system approximates to a rigid system, with almost the full arterial pressure ruling throughout, giving a greatly accelerated outflow from the veins. Finally the salivary pressure may rise much above the arterial pressure, but even then the circulation is not strangulated, because the membranæ propriæ limit the expansion of the alveoli.

In the brain and in the eye there exist no such membranæ propriæ, which can permit a difference of pressure between the secreted fluid and the blood. The ciliary processes are soft protoplasmic structures hanging in a bath of aqueous. The pressure of the blood within the capillaries and of the aqueous without must be one and the same. When fluid is forced in at a higher pressure than the arterial the capillaries are rendered bloodless. When the aqueous is allowed to escape, blood is forced in in increased volume to fill up the vacant space. The pressure within and without is no longer balanced, the dilated capillaries leak, and plasma passes out. Just as in the salivary gland the secretory pressure rules the circulatory pressure and tenseness of the gland, so the pressure of the ciliary processes rules the internal pressure of the eyeball. When the cells swell by imbibition of fluid and secrete the aqueous, droplet by droplet, there is less room for blood; hence the veins are narrowed and the pressure rises. It is clear that the secretory pressure produced by any cell in a vesicle of secretion may at any moment be greater than the capillary-venous pressure, but little by little, as the secretion is poured out, the two pressures, that of the aqueous and that of the blood, rise together. An immeasurably small difference of secretory pressure suffices to give the circulation of the aqueous. Absorption goes hand in hand with secretion, keeping the intraocular secretory pressure uniform. When the arterial pressure rises the intraocular pressure is raised, for the increased capillary-venous pressure is immediately transmitted to the aqueous. It goes without saying that a rise in the general arterial pressure would not necessarily produce an alteration of intraocular pressure, if the intraocular arteries were constricted at the same time.

At any moment there can be within the eye less blood and more tissue fluid, or more blood and less tissue fluid. Thus, within such encapsulated organs as the marrow of bone, the brain, and the eye, the volume of blood is not fixed. It can slowly change, increased by the transference of the tissue fluid or substance into the blood, diminished by transference of the plasma, fat, etc., to and turgescence of the tissues. The volume of blood within such organs is not affected by sudden variations of arterial pressure, for the expansion of the arteries is balanced by a corresponding shrinkage in the volume of the veins. The Monroe-Kellie doctrine is true only so far as regards these quick alterations.

Ophthalmic surgeons inform us that when the aqueous has leaked away the tension of the eyeball is not rapidly restored even though the wound is closed. Parsons* says "Prolonged pressure (applied) externally gradually squeezes out fluid from the eye, so that the pressure again becomes normal, but on removing the external pressure the tension remains subnormal until increased secretion produces compensation—a comparatively slow process. Hence after prolonged use of a pressure bandage the intraocular pressure may remain subnormal for some time." The secretion and vaso-dilatation are adjusted to suit the conditions which pertain. In the one case it is required that the tension be kept down so that the wound may heal, in the other case the contents of the eyeball are diminished by the pressure and become set at a lesser volume. It takes time for aqueous, blood, and tissue volume to return to the old volume when the pressure is removed. The capacity of the capillary vessels may be actually lessened by long applied pressure; both tissue and capillaries atrophy.

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* *Loc. cit.*, vol. 3, p. 1056.

Those who hold the mechanical theory of filtration of the aqueous assume that there is a hydrostatic difference of pressure on either side of the capillary wall. This assumption requires positive proof. It has never, in our opinion, been demonstrated that the pressure in the capillaries is higher than that of the surrounding tissue, or of the secretory fluid: neither in the case of the glomerular secretion of the kidney, nor in the case of lymph formation, and certainly not in the case of the cerebro-spinal or aqueous fluid. It has been assumed that if the arterial and venous pressures are raised, that in the capillaries must necessarily be raised also. This is not so. In the limbs the capillaries are emptied by the compressive action of the muscles and by change of posture. We have shown that in the dependent position of a limb there may be a high pressure in the arteries and, owing to gravity, a very considerable pressure in the large veins. But at the same time there may be no positive pressure in the capillaries after they have been emptied by muscular movement. Anyone can see that this is so, by holding his hand down and clenching his fist several times. Each time the capillaries are blanched, the valves in the veins permit this pumping action, and until the capillary bed is again filled to distension no positive pressure results.

The respiratory movements, the intestinal contractions and the movements of the skeletal muscles are all designed to pump the blood from the capillaries and keep a low pressure within them in any posture. Distension of the capillaries leads to a sense of discomfort which results in movement. In a case of hydrocephalus under Dr. Warner in the London Hospital the fluid was drawn off; after the introduction of the needle, we measured the pressure by the air index and counter-pressure method. It equalled about 20 cm. of water. On lifting up the head of the child the pressure of the fluid did not fall, as one might expect under the influence of gravity, but rose owing to the compressive action of the hands which raised the head, a bag of fluid. It is obvious in such a case that the cerebral-venous pressure and the fluid must be the same, and that as the fluid pressure is raised by compression of the head so must the cerebral-venous pressure rise *pari passu,* since it is not conceivable that the wall of the pial veins could support any part of the fluid pressure. On drawing off the fluid the pressure sank to atmospheric pressure and the soft wall of the skull lost all its tenseness and sagged in. So it remained for some hours, until, as the fluid was secreted, the tension gradually returned. In hydrocephalus we are dealing with an error of metabolism; the growth of the brain substance and the secretion of the cerebro-spinal fluid are not properly balanced. The pressure of the fluid and of the blood in the capillaries is the same; it is the relative secretory and growth properties of the brain and its membranes which are at fault. In cases of œdema of the legs we have measured the pressure of the fluid after introduction of a needle (for drainage). The pressure equalled about 50 cm. of water, and rose on lifting up the leg owing to the squeezing of the leg. The leg behaved, in this respect, like a bag of fluid, and the fluid and capillary-venous pressure must have been the same.

Ligation of the venæ vorticosæ, as shown by Adamük, produces a very large rise of intraocular pressure up to 90 mm. Hg. In albinos the local hyperæmia of the iris and the ciliary body can be seen. In this case, we maintain, the intraocular pressure rises because the capillary-venous pressure is raised almost to arterial pressure owing to the obstruction of the venous outflow. The fluid contents of the eye transmit this pressure equally in all directions, hence the intraocular pressure rises *pari passu* with the venous-capillary pressure. The rise of intraocular pressure, on the other hand, is ascribed by Parsons "primarily to the dilatation of the vessels, and secondarily to the high capillary pressure inducing increased transudation." "The anterior chamber becomes shallow and the filtration of fluid from the eye is thereby at first diminished, so that increased production is accompanied by diminished secretion." We find no explanation given as to how all these phenomena happen in an eye bounded by a rigid capsule. We recall the fact that the dead eye increases by only 7/1000 of its bulk when the intraocular pressure is raised from 19 to 70 mm. Hg. The living eye full of blood would increase much less. The shallowness of the anterior chamber is clearly due to the expression of aqueous into the substance of the cornea, spaces of Fontana, etc., under the influence of the high capillary-venous pressure which expands the blood-vessels.

Present day views of glaucoma are dominated by the idea that the aqueous fluid is a filtration product, and that the intraocular pressure is not the capillary-venous pressure but some other pressure controlled by the relation of filtration to absorption. From our point of view the pathology of the increased tension in acute glaucoma is plain. It is due to disordered metabolism which results in a greater imbibition force on the part of the disordered tissues and in arterial vaso-dilatation. Increased imbibition and blood supply in one part of the eye must be compensated for by narrowing of the veins and rise of vascular pressure. Hence the increased tension of the eyeball. Just as the tissues surrounding a bee sting, or a boil, swell and become tense, so does the eye become tense in an attack of acute glaucoma. The conditions are the same as in the salivary gland when the secretion is obstructed. The tissues confined by a capsule swell and the capillary-venous pressure is raised *pari passu* with the pressure of the imbibed or secreted tissue fluid. Thomson Henderson regards sclerosis of the cribriform ligament as the prime cause of glaucoma. The pathways of absorption of the aqueous are obstructed. This, he says, converts the circulatory system of the eye from an elastic to a rigid system.

"The free contact between aqueous and veins causes the intraocular pressure to be maintained at the normal intravenous level, as this is the lowest circulatory pressure. In glaucoma the contact is diminished, and the intraocular fluids, being contained in an unyielding capsule, act as a rigid volume which, operating through the tissues, compels the intraocular circulation to run in rigid lines. In a rigid system the outflow pressure is always higher than in a similar system of elastic tubes."*

We are not in complete agreement with this view, for the living tissues are not fixed mechanical structures. Moreover, supposing the cribriform ligament is sclerosed, the venous outflow no less remains patent, and for the aqueous there is the pathway of absorption through the iris veins. Obstruction of the absorption of aqueous *per se* can have no effect on intraocular pressure so long as the secretory pressure remains unchanged. Alterations in tissue metabolism undoubtedly are the primary cause. The obstruction of the natural circulation of the aqueous may possibly contribute to the alteration of tissue metabolism. However produced, it is increased imbibition which diminishes the venous content and raises intraocular tension.

Thomson Henderson has demonstrated, and we can confirm his demonstration, that "so long as the anterior chamber remains a closed space, the balance of fluid pressure between it and the vitreous chamber is perfect, so that if the vitreous pressure is raised, the aqueous pressure mounts to exactly the same height." We admit that fluid injected into the excised eye quickly flows out from the cut ends of the episcleral veins, but ceases to do so when the lens is pushed forward and the filtration angle blocked. We have pointed out, however, that the conditions in the living eye are entirely different, that the aqueous and venous pressure stand at the same level, and we have no proof that the angle acts as a filtration mechanism in it.

In our opinion surgical operations, *e.g.* iridectomy, relieve glaucoma by increasing the flow of tissue lymph through the inflamed part, thus promoting the healing process. The effect is just the same as that obtained on laying open a boil. The increased tension is not the cause of the trouble; that lies in the pathological condition which produces the tension. If the tissues of the eyeball become swollen by imbibition, the volume occupied by the blood in the veins is diminished, and the circulatory pressure and flow of blood is increased. If the imbibition proceed far enough and the arterial pressure be not increased by the compensatory action of the heart and vasomotor system, the circulation may become strangulated. Hence the utility of the hot fomentation which softens and of the surgeon's knife which cuts the confining structures and promotes the flow of blood and the outflow of plasma with its immunising power.

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* Thomson Henderson, *loc. cit.*, p. 135.

Arthur Thomson* has brought forward anatomical evidence for the view that the ciliary muscle opens the channels in the pectinate ligament. "They are kinked and compressed when drawn forward, opened and straightened when the scleral process is pulled back, thus imparting to them a valvular action." "In this way a negative pressure is established within Schlemm's canal into which fluids will pass along the line of least resistance. When the ciliary muscle, meridional and circular fibres, and sphincter of iris, cease to contract, the pectinate ligament by its inherent elasticity pulls the scleral spur forwards and outwards again, thus mechanically effecting the collapse of the inner wall of the canal of Schlemm against its outer wall, and so restoring it to the ordinary influences of the intraocular pressure." He supposes that filtration (outflow) of aqueous is maintained by this negative pressure and pumping action of the ciliary muscle, and that glaucoma may be caused by impairment of this action, *e.g.* by over-prolonged contraction of the hypertrophied circular fibres in the hypermetropic eye. We conclude that Arthur Thomson accepts the received explanation of glaucoma, viz., that it is due to obstructed absorption of the aqueous, and defines a mechanism which he believes acts as a valved pump and furthers the exit of the aqueous.

Holding as we do that the inflow of the aqueous is governed by secretion, that is, by the vital action of the cells lining the ciliary processes, and that it is not a filtration fluid dependent on the blood pressure, we do not recognise the need for any such mechanical pump controlling the outflow. We believe that absorption is no less controlled by the endothelial cells which separate the fluid from the veins. Both secretion and absorption we maintain are functions dependent on the living energy of the cells. We agree, however, with Thomson Henderson when he says that "the ciliary muscle by its contraction opens up the channels which allow aqueous to pass from in front of the lens to the circumlental region." This, we believe, is the purpose of the structural arrangements examined by Arthur Thomson. The transference of fluid is essential to the act of accommodation.

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* 'The Ophthalmoscope,' London, July, 1911.

In text-book discussions on the mechanism of accommodation, little or no attention is paid to the behaviour of the aqueous at the moment when the anterior surface of the lens changes its shape. The ciliary muscle is described as fixed at the junction of the cornea and sclerotic and inserted into the choroid; it is supposed to pull up the choroid so as to relax the tension of the suspensory ligament when the lens by virtue of its elasticity becomes more convex. From whence, we may ask, does the lens obtain the "virtue of its elasticity"? An excised lens has an almost fluid exterior and a much stiffer glue-like core. The tension of the choroid is due to the intra-ocular pressure, this depends on the secretory pressure of the aqueous, which in its turn determines the circulatory pressure in the eyeball. The pressure in the aqueous, in the vitreous, in the lens, and in all the capillary-venous networks within the sclerotic-corneal envelope is one and the same. While the vitreous is enclosed in the hyaloid membrane, the membranes of Descement, of Bruch, and the basement membrane of the iris and ciliary processes form one continuous membrane, which, we believe, acts like the membranæ propriæ of the alveoli of the salivary gland—a kind of semi-permeable membrane, permitting and confining the secretion of aqueous. The fluids of the eye distribute the pressure equally in all directions. The manometric observations of Hess and Heine show that no change of intra-ocular pressure accompanies the act of accommodation. No change in the fulness of the retinal vessels has been observed in the eye of man on accommodation.

On the other hand, Arthur Thomson cites observations made by Ulbrich on a case of partial coloboma of the iris, where the aperture was closed by a delicate membrane which served as a natural and very sensitive manometer. Although dilatation and contraction of the pupil under the action of light did not alter the position of the membrane, yet during accommodation the membrane sank, the extent to which it receded being roughly proportional to the amount of accommodation. Under eserine this reaction was intensified whilst atropine, on the contrary, annulled it entirely. Now, in order that the lens may become convex forwards it is necessary that fluid should be transferred from the anterior chamber in front to the region surrounding the circumference of the lens. Helmholtz recognised this, and supposed that the angle of the anterior chamber was deepened to make room for the displaced aqueous. Thomson Henderson controverts this on the evidence derived from the structure of eyeballs which have been properly fixed, and points out that the cribriform ligament cannot be pulled backwards so as to cause an anatomical deepening of the angle. The ciliary muscle, however, by its action opens up the supra-choroidal space and the meshes of the cribriform ligament. "The circular sphincter fibres of the ciliary muscle move inwards on accommodation and, straightening out the innermost fibres of the cribriform ligament, allow the passage of aqueous into the supra-choroidal space." Thomson Henderson has traced this passage by injections of Indian ink. We maintain that the instantaneous transference of aqueous to and from the front and the circumference of the lens is the essential part of the act of accommodation. Without such transference the sphincter action of the ciliary muscle could not take place: the semi-fluid exterior part of the lens confined in its capsule bulges forwards in proportion as the aqueous fluid is transferred from in front to the circumlental region. This is the function which is subserved by the structure of the ciliary region and the ciliary muscle, and the anatomical arrangements required to be re-examined with this function in view. By the perfect-balanced action of the intraocular fluids pressure is instantly transmitted, and the fineness and celerity of the movements of accommodation are made possible. We suggest that certain of the longitudinal fibres and the circular fibres are antagonistic in action, and balance the to-and-fro movements of the fluid. Thomson Henderson has suggested an antagonism of action, but in relation to the tension of the suspensory ligament. The anatomical arrangement of the ciliary muscle figured by Henderson would equally well subserve the movement of fluid to and from the circumlental region. The manometric method shows no change of pressure on accommodation because the lens bulges forwards in proportion as the circumlental region swells. The delicate membrane observed by Ulbrich gave evidence of the sudden pull of the muscle and its action on the aqueous fluid. We opine that the shaking of the lens observed by Hess results from the transference of fluid to the circumlental region. This fluid affords a waterbed on which the lens can shake.

In confirmation of the view which we have put forward we quote the observation made by Beer, that accommodation through stimulation of the ciliary muscle is no longer possible when a hole is cut into the posterior wall of the eye so that the counterbalancing pressure of the vitreous is *nil.* The sphincter action of the ciliary muscle depends on the counterbalancing influence of the intraocular pressure; the pressure of the vitreous gives an equable support behind, while the ciliary muscle, by its sphincter action, widens the meshes of the ciliary body and cribriform ligament, and draws aqueous into the circumlental region.

Summary.

(1) The intraocular pressure is 35–65 mm. Hg in cats and in dogs under ether. To the finger, the tension of the human eye seems to be about the same as in these animals.

(2) The tension varies with the arterial pressure; a residual pressure of some 13–18 mm. Hg remains for a short time after the heart beat has stopped.

(3) The tension is primarily due to the secretory action of the cells which line the ciliary processes; this secretory pressure regulates the capillary-venous pressure pertaining in the eyeball; the pressure of the aqueous and the capillary-venous pressure are always one and the same. Sudden abolition of this equality of pressure by escape of the aqueous causes congestion of the iris and ciliary body, and hæmorrhage then results from the iris when the blood pressure is raised.

(4) The circulatory conditions in the eye resemble those in the intracranial cavity, with the exception that the intraocular is much higher than the intracranial pressure, and therefore is not affected by changes in the general venous pressure.

(5) When the intraocular pressure is raised by injection of Ringer's solution into the anterior chamber, the circulation through the eyeball continues until the intraocular pressure just exceeds the arterial pressure.

(6) It is suggested that the increased tension in glaucoma is due to increased imbibition and secretion of fluid, resulting from an altered metabolism of the ocular tissues, leading to compression of the veins and to a rise in the capillary-venous pressure, and therefore intraocular pressure. The operative relief of glaucoma depends not on the relief of the tension *per se*, but on the increased transudation of tissue lymph with its immunising properties. Acute glaucoma resembles an inflammatory condition in any other part of the body, *e.g.* a boil.

(7) The essential factor in the act of accommodation is the transference of the aqueous from the front of the lens to the circumlental region, which allows the forward expansion of the fluid exterior part of the lens.

The Oxydases of Cytisus Adami.

By Frederick Keeble, Sc.D., Professor of Botany, University College, Reading, and E. Frankland Armstrong, Ph.D., D.Sc.

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The graft-hybrid *Cytisus Adami*—that classic of botanical speculation and research—is one of the wonders of the vegetable world. Every year the trees, which have been propagated vegetatively from the original creation of the French gardener Adam, may be seen to bear blossoms of three kinds, buff, yellow, and purple. The yellow flowers are identical with those of the common laburnum (*Cytisus laburnum*), the purple resemble those of *Cytisus purpureus,* and the buff coloured flowers appear to combine the characteristic features of *C. laburnum* with those of *C. purpureus.*

The history of the origin of *C. Adami* has been told repeatedly (*cf.* Darwin, 1888). There is good ground for believing that it was produced by budding *C. purpureus* on *C. laburnum.* The plants raised as a result of the operation were—according to Darwin's account—distributed originally as *C. purpureus,* and it was not till later, when they began to exhibit their diversity of flower, that they were described as graft-hybrids. Notwithstanding the attention that has been given to the subject, the nature of the union between the two species remained obscure for many years.

Recently the brilliant researches of Baur (1909) and Buder (1910) have led the former author to formulate an hypothesis which has the signal merit of presenting a precise and diagrammatic picture of the biological construction of *C. Adami.*

On this hypothesis, the graft-hybrid is to be regarded as a periclinal chimera; that is to say, it is a dual organism composed externally of one species and internally of another. The contribution of *C. purpureus* to the chimera consists of a superficial single-layered epidermis; the rest of the body of *C. Adami* is built up of tissues contributed by *C. laburnum.* The hypothesis, which is supported by the results of investigations into the comparative anatomy of the component species and the graft-hybrid, and also by analogy with other graft-hybrids, permits of a postulation of the modes of origin of the several kinds of flowers borne by *C. Adami.* Thus, branches of the graft-hybrid in which the periclinal duality is maintained bear flowers with a purple epidermis and yellow sub-epidermal cells. The combination results in a buff colour. Those branches which, for some reason or other as yet unexplained, are produced solely by the sub-epidermal laburnum cells bear yellow flowers of the laburnum type. The purple flowered branches arise exclusively from epidermal cells, and are, therefore, similar to those of *C. purpureus.*

The investigations, the results of which are now to be described, were undertaken with two objects. In the first place, they were designed to test the validity of Baur's hypothesis, and, in the second place, to determine whether the results of an examination of the distribution of oxydases in *C. Adami* and its component species would confirm the hypothesis, to which our previous work (Keeble and Armstrong, 1912) has led us, that oxydases may migrate from one part of a plant to another.

We may say at once that the results of our investigation of the distribution of oxydases in *C. Adami* serve to confirm the general truth of Baur's conclusions and to support the thesis that oxydases may migrate from cell to cell.

The methods which we employ for determining the distribution of oxydases in plant-tissues are described in our former contribution.

The results obtained by the use of benzidine are as follow: Petals of *C. purpureus* treated with an alcoholic solution of benzidine and incubated at 37° C. for half an hour are first decolorised and then give a direct oxydase reaction, that is one which declares itself without the addition of hydrogen peroxide. The reagent produces a grey to bluish-brown coloration of the general surface of the petals, more marked in the marginal region, and a similar but deeper coloration of the veins. In other words, *C. purpureus* contains an epidermal oxydase and also a bundle oxydase.

The petals of the purple (magenta) flowered branches of *C. Adami*—which branches are, on Baur's hypothesis, pure *C. purpureus*—give similar, albeit somewhat fainter, reactions, thus indicating that the oxydases of the purple branches of *C. Adami* are identical in kind and distribution with those of *C. purpureus.* When treated with benzidine the typical buff-coloured flowers of *C. Adami* do not become completely decolorised. This is owing to the fact that they contain, in addition to an anthocyanic epidermal pigment, a yellow pigment of the carotin type, which lies, in both *C. Adami* and *C. laburnum,* in the sub-epidermal cells. Nevertheless, despite the fact that the carotin pigment is present, it is easy to determine, either by direct inspection or by microscopic examination of surface sections of the petals, that the buff-coloured flowers of *C. Adami* contain epidermal oxydase. In this respect *C. Adami* differs from the yellow *C. laburnum* and from the laburnum-like flowers of the yellow-flowered branches of *C. Adami,* neither of which contains epidermal oxydase. We conclude, therefore, that the epidermis of *C. purpureus* contains an oxydase, that this oxydase is present also in the epidermis of *C. Adami*—both in the buff-coloured typical *C. Adami* flowers and in the purple flowers borne on *C. Adami;* but that it is absent from the epidermis of *C. laburnum* and from that of the yellow flowers of *C. Adami.*

Although, as just indicated, the petals of *C. purpureus* and *C. Adami* give similar epidermal oxydase reactions they differ from one another with respect to the mode of reaction of their deeper tissues. For whereas the petals of both *C. purpureus* and of purple-flowered branches of *C. Adami* give, with benzidine alone, a well-marked reaction for bundle oxydase, the vascular bundles of the buff petals of *C. Adami,* those of the yellow petals of the laburnum-like branches borne on the graft-hybrid and those of the petals of *C. laburnum* give no direct oxydase reaction. In the three last-mentioned cases it is only after the addition of hydrogen peroxide that the bundle oxydase reaction is developed. Whence it is to be concluded that oxydase is present in the tissues which accompany the veins of the petals of *C. purpureus* and purple-flowered branches of *C. Adami,* and that peroxydase is present in the corresponding tissues of the buff and yellow flowers of *C. Adami,* and in those of the common laburnum.

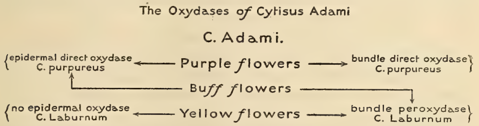
A closer examination of the brown bundle oxydase reaction produced by benzidine shows that it is most marked in *C. purpureus,* fairly marked in the purple-flowered branches of *C. Adami,* and that it does not occur in the buff flowers of typically *Adami* branches. This is in accord with the differences in pigmentation of the three flowers. For in the living flowers of *C. purpureus* lines of darker purple (magenta) accompany the veins of the petals. Similar though less well-marked magenta lines run with the veins of the purple flowers of *C. Adami,* whereas there are no such lines of magenta colour along the veins of the buff *C. Adami* petals. We conclude, therefore, that in flowers the oxydases which accompany the veins (bundle oxydases) co-operate with those contained in the epidermal cells to produce the pigment of those cells. If this view be accepted, the failure of *C. Adami,* although its epidermis is that of *C. purpureus,* to produce flowers of as deep a purple as those of *C. purpureus,* is intelligible; for the vascular system of *C. Adami* is derived from the *C. laburnum* component, and in that species the vascular tissues of the flower—though they contain peroxydase—possess no direct oxydase, and it is therefore reasonable to suppose that they cannot contribute to pigment formation in the epidermis.

If, further, we assume, and it is an assumption for which grounds are not altogether lacking, that oxydases may travel through the vascular tissues, it is possible to account for the fact that the purple pigment of the flowers of *C. Adami* is generally paler than that of the flowers of *C. purpureus.*

The former receive only the oxydase of the *purpureus* branch, the latter have at their disposal contributions from the whole plant.

The conclusions based on the results of treating petals with benzidine are confirmed by those obtained with α-naphthol. For although this reagent produces no definite epidermal reaction,* it gives rise to distinct bundle oxydase reactions in the flowers of *C. purpureus* and purple *C. Adami*. On the other hand, α-naphthol gives no direct oxydase reactions, neither in the flowers of *C. laburnum*, typical *C. Adami*, nor in those of the yellow branches of *C. Adami*. In these last-mentioned forms α-naphthol and hydrogen peroxide produce the characteristic colour-reaction, whence it is to be concluded that they contain peroxydase.

As indicated in the accompanying diagrammatic representation of the results described above, the accuracy of Baur's representation of *C. Adami* as a periclinal chimera is confirmed by a study of the oxydases of the flowers of the component species and of the synthetic form. Whether, however, the growing points of *C. Adami* consist always of a single-layered mantle of *C. purpureus* cells enveloping cells solely of the *C. laburnum* type must remain for the present uncertain.


The Oxydases of Cytisus Adami
C. Adami.
epidermal direct oxydase C. purpureus
Purple flowers
bundle direct oxydase C. purpureus
Buff flowers
no epidermal oxydase C. Laburnum
Yellow flowers
bundle peroxydase C. Laburnum

It is not too much to suppose that, if the migration of oxydases is finally proved and shown to be of general occurrence, considerable light will be thrown on the nature of the, at present, puzzling differences in form between the flowers of *C. Adami* and those of the component species, and in particular it may help to elucidate the causes of differences which exist between the flowers of *C. purpureus* and those of *purpureus* branches of the graft-hybrid.

The facts now to be mentioned appear to point distinctly to a migration of oxydase from cell to cell. The most elegant part of Baur's demonstration of the peri-chimeral nature of *C. Adami* has reference to small groups of cells containing red (anthocyan) pigment which occur in the neighbourhood of the median line on the lower parts of the standards of flowers of *C. Adami*. These patches of pigmented cells—described sometimes as honey-guides—though visible on the surface of the intact flower are situated in the sub-epidermal layer. They must, therefore, as Baur points out, be formed by the *C. laburnum* component of the chimera. Examination of the flowers of *C. laburnum* shows the presence of these red-brown or chocolate patches on the standards and hence they supply Baur with a convincing argument that the sub-epidermal tissues of *C. Adami* are derived from the laburnum (*C. laburnum*).

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* See Keeble and Armstrong, *loc. cit.*

Now microscopic examination of transverse sections across the patches of pigment cells on the standard of a flower of *C. Adami* shows at once that the cells, of *purpureus* origin, which overlie the sub-epidermal pigmented cells of *laburnum* origin, are always more deeply pigmented than are the neighbouring epidermal cells, which have no anthocyan-containing cells immediately beneath them. In other words the deeper pigmentation of the epidermal *purpureus* cells coincides with the distribution of soluble (anthocyan) pigment in the underlying and contiguous *laburnum* cells. In other parts of the petals, the *laburnum* cells contain only yellow carotin and there the purple colour of the epidermal cells remains faint.

Since, by the nature of the case, pigment-containing cells are impermeable to their soluble pigments, it seems very probable that the heightened colour of the epidermal cells which are contiguous with the red patches of sub-epidermal cells is due to a migration of oxydase from the sub-epidermal, *laburnum* cells, to the epidermal, *purpureus* cells.

It might perhaps be urged that the paleness of the epidermal cells which overlie the yellow tissues is due to some inhibition of oxydase on the part of the latter. This, however, does not appear to be the case. For as we have shown (*loc. cit.*) it is possible in other flowers (*Primula sinensis*) to destroy the inhibitor of oxydase which exists in dominant white flowers by treating the petals with hydrogen cyanide. This reagent, however, when applied to flowers of *C. Adami* does not exert any influence on the strength of the oxydase reactions subsequently obtained. Hence we conclude that the yellow cells do not exercise an inhibitory effect on the pigment-forming action of the oxydase in the epidermal cells.

Finally, we would point out that the hypothesis of oxydase migration, not only from sub-epidermal to epidermal cells, but also from the vascular tissues to the epidermis, is in conformity with what is known with respect to the variable behaviour of many florists' flowers. Thus, in certain seasons wallflowers, and in all seasons stocks, tulips, etc., produce flowers which "break." For example a pure strain of self-coloured wallflowers may become blotched or flaked. There is evidence in the case of wallflowers that this breaking has no relation with genetical constitution, for a strain which "breaks" badly one year may behave in a perfectly normal manner and produce only self- (uniformly) coloured flowers in the following year. Moreover growers associate the phenomenon of breaking of wallflowers with some abnormality in the development of the "wood." Again, in the case of stocks it is correlated with a blotched form of leaf: the plants which bear flaked flowers possess leaves with irregular light green patches. It may be that certain tissues of the vascular system are to be regarded as secretors of oxydases which have among other functions the formation of flower pigments and that, with the oxydase-secreting mechanism out of gear, the uniformity of colour of the flowers is destroyed.

To this subject, however, we hope to return.

In conclusion we wish to express our thanks to Mr. W. Watson, Curator of the Royal Gardens, Kew, and to others for supplying us with material for this investigation and have to acknowledge the assistance which we have derived from a grant by the British Association.

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Some Conditions Influencing Nitrogen Fixation by Aërobic Organisms.

By W. B. Bottomley, M.A., Professor of Botany, King's College, London.

(Communicated by Dr. J. Reynolds Green, F.R.S. Received May 30,—
Read June 27, 1912.)

The fixation of nitrogen by *Azotobacter* and *Pseudomonas* depends upon the presence of a fermentable carbohydrate as a source of energy. Although a large number of carbohydrates can be utilised by these organisms there is a considerable variation in their efficiency, and for effective fixation it is necessary to work with a sugar specially suitable for the organism under investigation. It is generally recognised that mannite is the most efficient kind for *Azotobacter* and maltose for *Pseudomonas.* Unfortunately mannite is practically useless for *Pseudomonas* and maltose for *Azotobacter*. Further it was found when working with a mixed culture of *Azotobacter* and *Pseudomonas* that the mixture of carbohydrates (mannite and maltose) was not satisfactory. It was desirable, therefore, to obtain a carbohydrate which would be equally efficient as a source of energy for both organisms. This has been obtained in dextrin.

In order to compare the influence of dextrin with mannite and maltose upon *Azotobacter* and *Pseudomonas* respectively, six series of Erlenmeyer flasks, four flasks in each series, were arranged. All the flasks contained 100 c.c. of a nutrient solution consisting of di-potassium phosphate 0·2 grm., magnesium sulphate 0·02 grm., basic slag 0·4 grm., in 100 c.c. distilled water.

The series were treated as follows:—A received 1 grm. dextrin; B, 1 grm. maltose; C, 1 grm. dextrin; D, 1 grm. mannite; E, 2 grm. dextrin; F, 1 grm. maltose and 1 grm. dextrin.

A and B were each inoculated with 1 c.c. of a pure culture of *Pseudomonas,* C and D with 1 c.c. of a pure culture of *Azotobacter,* and E and F with 2 c.c. of a mixed culture (1 c.c. of *Pseudomonas* and 1 c.c. of *Azotobacter*). In each series flask 1 was autoclaved immediately after inoculation and served as a control; flask 2 was used for testing the carbohydrate; flasks 3 and 4 were analysed for amount of nitrogen fixation. All the flasks were incubated at 26° C. until the carbohydrate was completely used up as shown by flasks 2. Nitrogen determinations were then made and the following results obtained:—

Series.	Contents.	Total nitrogen.	Gain in N per grm. carbohydrate.	Average gain.
		mgrm.	mgrm.	mgrm.
A	*Pseudomonas* on 1 grm. dextrin—			
	1	0·32		
	3	2·72	2·40	2·48
	4	2·88	2·56	
B	*Pseudomonas* on 1 grm. maltose—			
	1	0·28		
	3	2·96	2·68	2·54
	4	2·68	2·40	
C	*Azotobacter* on 1 grm. dextrin—			
	1	0·64		
	3	7·04	6·40	6·32
	4	6·88	6·24	
D	*Azotobacter* on 1 grm. mannite—			
	1	0·32		
	3	7·20	6·88	6·96
	4	7·36	7·04	
E	*Azotobacter* + *Pseudomonas* on 2 grm. dextrin—			
	1	0·32		
	3	15·05	14·73	14·89
	4	15·37	15·05	
F	*Azotobacter* + *Pseudomonas* on 1 grm. maltose + 1 grm. mannite—			
	1	0·70		
	3	12·28	11·58	11·86
	4	12·84	12·14	

From the above figures it is evident that dextrin is about as efficient as maltose for *Pseudomonas*, and as mannite for *Azotobacter*, whilst for a mixed culture of the two organisms it is much more effective than a mixture of maltose and mannite.

Another difficulty encountered in working with mixed cultures was the neutralisation of the nutrient medium. The neutralisation agents generally employed are calcium carbonate for *Pseudomonas*, and sodium hydrate for *Azotobacter*. In some preliminary experiments with various neutralising agents it was found that basic slag gave excellent results with both organisms, not only effectively neutralising the medium but also increasing nitrogen fixation. This tonic effect of basic slag is probably due to the presence of iron and manganese.

Krzemieniewski* has shown that humus stimulates the fixation of nitrogen by *Azotobacter* in a remarkable manner. Later it was pointed out by Remy and Rösing† that this stimulating effect is due to the presence of iron in the humus. As these experiments had reference only to *Azotobacter* an investigation was made on the effect of humus and basic slag (which contains iron) on the fixation of nitrogen by pure cultures of *Pseudomonas.*

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* 'Bull. Acad. Sci. Cracovie,' 1908.

† 'Centrbl. f. Bakt.,' 1911, vol. 30.

Three series of four flasks each were arranged. Each flask contained 100 c.c. of culture solution containing 1 per cent. dextrin, 0·2 per cent. di-potassium phosphate and 0·02 per cent. magnesium sulphate. Each flask was inoculated with 1 c.c. of a pure culture of *Pseudomonas* from the root nodules of *Phaseolus vulgaris.* Series A received 1 per cent. of chalk; Series B, 0·03 per cent. of potassium humate; and Series C, 0·4 per cent. basic slag (containing lime 47 per cent., phosphoric acid 18·88 per cent., and iron 6·89 per cent.).

The flasks were inoculated at 26° C. until all the dextrin had disappeared. Nitrogen determinations then gave the following:—

Series.	Contents.	Total nitrogen.	Gain in N. per grm. carbohydrate.	Average gain.
		mgrm.	mgrm.	mgrm.
A	Culture + 1 per cent. chalk—			
	1	0·28		
	3	2·26	1·98	1·95
	4	2·19	1·91	
B	Culture + 1 per cent. chalk + 0·03 per cent. potassium humate—			
	1	0·55		
	3	3·67	3·12	3·19
	4	3·81	3·26	
C	Culture + 0·4 per cent. basic slag—			
	1	0·28		
	3	3·39	3·11	3·04
	4	3·25	2·97	

The above table indicates that for cultures of *Pseudomonas* basic slag is a much more effective neutralising agent than chalk, and that its stimulative effect on nitrogen fixation is very nearly equal to that of humus.

From the two sets of experiments described above it is evident that a simple and effective nutrient medium for both *Azotobacter* and *Pseudomonas* is obtained by adding to distilled water 1 per cent. of dextrin, 0·2 per cent. of di-potassium phosphate, 0·02 per cent. magnesium sulphate and 0·4 per cent. basic slag.

The Intensity of Natural Selection in Man.

By Karl Pearson, F.R.S.

(Received June 4,—Read June 27, 1912.)

(1) In a paper[*] communicated to the Royal Society in 1899, and later in greater elaboration published in 'Biometrika,'[†] 1901, it has been shown on the basis of the inheritance of longevity that the selective death-rate in man amounted to at least 60 per cent. to 80 per cent. of the total death-rate. The matter has been recently reconsidered by Prof. Ploetz,[‡] who, dealing with material wholly different from that of Beeton and Pearson came to similar conclusions. The point is a very vital one, for, combined with: (i) the heredity of physical and mental characters in man,[§] and (ii) the demonstration that the longer-lived have more offspring,[||] we reach a definite knowledge that Darwinism does apply, and very intensely applies, even to man under civilised conditions.

The difficulty of a direct investigation of the problem lies in securing uniformity of environment. We have to demonstrate that when under the same environment there is a heavier death-rate among a given group of human beings, then among the survivors of this group in a given later period the death-rate will be lessened. Now each group of individuals we attempt to deal with has its own environment, and if that is a bad environment we should expect to find a heavy death-rate both at the earlier and later periods; this obviously must obscure the action of natural selection. For example in districts with a high infant mortality we might expect a high child mortality, say deaths from two to five years of life, because a bad environment sends up the intensity of both. The correlation between deaths in the first year of life (0—1) and in the next four years of life (1—5) for a given district will certainly be positive if no correction be made for varying environment. Quite recently this matter has been discussed by determining the correlation between the ages 0—1 and 1—5 in the administrative counties of England and Wales.[¶] As (*a*) the group 0—1 was not followed to 1—5, but the deaths in these age-groups for the *same* years were dealt with, and (*b*) no allowance whatever was made for the differential environment of the administrative counties, it is difficult to find any real bearing of the data on the problem of natural selection in man.

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[*] 'Roy. Soc. Proc.,' vol. 65, p. 290, *et seq.*

[†] Vol. 1, p. 50, *et seq.*—especially pp. 74–5.

[‡] 'Archiv für Rassen- u. Gesellschafts-Biologie,' 1909, vol. 6, p. 33.

[§] "On the Laws of Inheritance in Man. I.—Inheritance of Physical Characters," 'Biometrika,' vol. 2, p. 357; II.—"On the Inheritance of the Mental and Moral Characters in Man," 'Biometrika,' vol. 3, p. 131.

[||] "On the Correlation between Duration of Life and Number of Offspring," 'Roy. Soc. Proc.,' vol. 67, p. 159.

[¶] 'Local Government Board Report,' Cd. 5263.

The only method by which data for different districts can be compared is by endeavouring to fix the nature of the environment. We want to know whether under a constant environment the correlation between the death-rates of infancy and of childhood is positive or negative. Dr. E. C. Snow,* working on both English and Prussian data, finds, using a variety of criteria of sameness of environment, that when this factor is allowed for the correlation between infantile and child death-rate is negative, and substantially negative. In other words, within the same group under the same environment, the greater the infantile death-rate, the less is the death-rate among the survivors; that is to say, the physically stronger members of the group are those which survive the ordeal.

Dr. Arthur Newsholme in his recent paper, "National Importance of Child Mortality,"† has asked the question (p. 332):—"Is it certain that a lower infant mortality will produce a survival of an increased proportion of physically inferior children?" and he reaches the conclusion that:—"A high infant death-rate in a given community implies in general a high death-rate in the next four years of life, while low death-rates at both age-periods are similarly associated" (p. 334). "So far from any weeding out of the weaklings being manifest, the counties with a high infant mortality have a death-rate which is relatively higher still in the next four years of life." As evidence for this he cites the correlation between infantile and child death-rates for the same period, and the life-tables for Healthy Districts and for all England and Wales. These life-tables, however, show, as I indicate below, that a heavier infantile mortality has actually a lower child mortality associated with it. It appears to me that the non-allowance for differential environment renders Dr. Newsholme's reasoning insecure.

It is not generally realised that the infantile mortality in England and Wales has not been falling but steadily rising since the restriction in size of families. On the other hand, the child mortality has been steadily falling. The numbers provided by the Registrar-General's Life-Tables‡ are:—

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* "The Intensity of Natural Selection in Man," 'Drapers' Company Research Memoirs,' Dulau & Co., 1911.

† 'Journal of the Royal Sanitary Institute,' 1910, vol. 31, p. 334.

‡ 'Supplement to the 65th Annual Report,' 1891—1900, Part I, pp. xlviii—l, London, 1907.

English Life-Tables.

Period.	Males. Deaths per 1000 individuals.		Females. Deaths per 1000 individuals.	
	0—1 years.	1—5 years.	0—1 years.	1—5 years.
1838—1854.........	163·50	134·73	134·71	132·60
1871—1880.........	158·58	127·58	128·73	124·70
1881—1890.........	161·04	104·26	131·13	98·55
1891—1900.........	171·86	94·01	140·66	89·83

Now, these data are the most considerable we can get and they involve all environments in this country. If we might suppose the environment of the country as a whole to have remained constant, we could only conclude that it is certain that a high infant death-rate in a given community implies in general a low death-rate in the next four years of life.

It is difficult to realise how these data have come to be overlooked. It is clear from them that the improved environment of the last 30 to 40 years has not effected any improvement in the infantile death-rate. Either motherhood is less efficient, or the quality of the infants has degenerated, and they can resist worse a better environment. It seems *a priori* unreasonable to suppose the improvement in child mortality wholly due to an improved environment which has produced no effect on infant mortality; it is *a priori* not unreasonable to assume that some of the improved child mortality is due to increased infantile mortality. Can we to any extent determine these proportions?

(2) There are two preliminary points to be considered in this matter. In the first place the actuarial calculation of a life-table is not based on following a given group of persons through life, and determining how many die at each age. At first sight, therefore, our objection (*a*) above applies to approaching the problem of natural selection in man from the life-table standpoint. But a little consideration will show that it is far more justifiable than dealing with local districts in the *same* year. We have in the first place an enormous mass of material—the whole country—and the results are taken for 10 or 16 years. We thus obtain a stable community representing the average of what is taking place in a community gradually changing throughout the epoch. The general experience of actuaries who use such tables for all purposes indicates that the death-rates calculated in this manner for various groups of individuals closely represent what occurs, if the same group be followed through life—the accidental excesses or defects, scarlet fever or measles epidemics, etc., are averaged out when a whole

country and considerable periods are dealt with. I think we may say with fair certitude, for example, that of 1000 male children born in the period 1871–1880, 158·58 would die in the first year of life, and of their survivors, 841 in number, $(841/1000) \times 127{\cdot}58$ would die in the second to the fifth years of life. Further it must be remembered that owing to the period (10 and 16 years) covered, the arrays born in each year but the last one or two have contributed their survivors to die in the whole or part of the child period 1—5.

My next point is the problem of environment. We are told that the environment has been continuously improving during the last 40 to 50 years, and if we ask for a measure of it we are very rightly referred to the falling death-rate, which remained stationary until 1866, and then has been falling in a remarkable way ever since. To correct therefore for continuously improving environment we may take something closely associated with the death-rate; it occurred to me that expectation of life would be an excellent measure of this change of environment. In order, however, to introduce no spurious correlation by taking the expectation of life at birth, which would include the influence of the very mortalities I am dealing with, I have taken the expectation of life at the age of 6 years as the factor by which to correct for the secular change in environment.*

Of course, I very fully realise the audacity of determining correlations from four life-tables only,† but it must be remembered that each one of my figures is based upon a population of millions, and even if we considered our total number of observations four only, the fundamental partial correlations are still immensely significant as compared with their probable errors. Further, I shall show that calculated and observed results are in agreement.

Tables I and II give the data. Underneath the tables I have placed the chief statistical constants; i = infantile death-rate (0—1 years) in deaths per thousand; c = death-rate of children (1—5 years) in deaths per thousand; e = expectation of life in children aged 6.

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* Should it be said that the expectation of life at six years of age is influenced by the mortality which occurs in the first five years, this in itself would be to admit that the death-rate is truly selective, the very point we have set out to prove, as against those who hold that the infantile death-rate is not selective.

† We have to bear in mind the vast amount of work involved in computing a table of this kind, and recognise that in calculating 14 life-tables in four series the General Registry Office has achieved a great task.

Table I.—Males.

Years of life-table.	*i.*	*c.*	*e.*
1838—1854	163·50	134·73	49·39
1871—1880	158·58	127·58	50·38
1881—1890	161·04	104·26	52·19
1891—1900	171·86	94·01	52·88
Means	$m_i = 163·744$	$m_c = 115·145$	$m_e = 51·21$
S. D.'s	$\sigma_i = 4·9992$	$\sigma_c = 16·6085$	$\sigma_e = 1·3920$

From these data we further deduce:—

$$r_{ic} = -0·6359$$
$$r_{ie} = +0·5620$$
$$r_{ce} = -0·9941$$

These lead to the partial correlation coefficient of infantile and child death-rates for constant environment

$${}_{e}r_{ci} = -0·8605 \pm 0·0875.$$

Notwithstanding the small number of life-tables dealt with this is most substantially significant. With our measure of constant environment, every increase of the infantile death-rate is accompanied by a marked decrease in the death-rate of the survivors in childhood.

The multiple regression equation is

$$\begin{aligned} c &= 115·145 - 0·3748\,(i - 163·744) - 11·0038\,(e - 51·21) \\ &= 740·021 - 0·3748\,i - 11·0038\,e. \end{aligned} \qquad (1)$$

Thus for constant environment, *i.e.* e constant—

Increase in child death-rate = −0·3748 (increase in infantile death-rate).

Or, looked at in another way—

Percentage decrease in child death-rate = 0·533 (percentage increase in infantile death-rate).

In other words if you increase the infantile death-rate by 10 per cent. you decrease the child death-rate by 5·3 per cent.

The approximation of the method is indicated by the following table:—

Period.	Observed *c.*	Calculated *c.*	Δ.
1838—1854	134·73	135·26	+0·53
1871—1880	127·58	126·21	−1·37
1881—1890	104·26	105·38	+1·12
1891—1900	94·01	93·73	−0·28

The results are in far better accord than we might have anticipated, and we see from these male data that—

(i) Our environmental measure appears to be justified by its close correlation (0·994) with the child death-rate.

(ii) Notwithstanding this the infantile death-rate is fairly closely correlated with the environmental measure in the *negative* sense.

(iii) The infantile and child death-rates, correcting for the environmental factor, are very substantially negatively correlated.

We may now turn to the female data—

Table II.—Females.

Years of life-table.	*i.*	*c.*	*e.*
1838—1854	134·71	132·60	50·00
1871—1880	128·73	124·70	52·56
1881—1890	131·13	98·55	54·35
1891—1900	140·66	89·83	55·18
Means	$m_i = 133·81$	$m_c = 111·42$	$m_e = 53·02$
S. D.'s	$\sigma_i = 4·4922$	$\sigma_c = 17·7251$	$\sigma_e = 1·9853$

From these data we further deduce

$$r_{ic} = -0·5080$$
$$r_{ie} = +0·3042$$
$$r_{ce} = -0·9507$$

The partial correlation coefficient between infantile and child death-rates is

$$_{e}r_{ic} = -0·7399 \pm 0·1459.$$

This is again substantial and significant. It means again that by fixing the environment as far as lies in our power, we have increased the negative correlation between infantile and child death-rates.

The multiple regression formula is now

$$\begin{aligned} c &= 111·42 - 0·9514\,(i - 133·81) - 7·8335\,(e - 53·02) \\ &= 654·06 - 0·9514\,i - 7·8335\,e. \end{aligned} \qquad (2)$$

Clearly for a constant environment—

Increase in child death-rate = −0·9514 (increase in infantile death-rate).

Or, in other words—

Percentage decrease in child death-rate = 1·1424 (increase in infantile death-rate).

Thus in the case of females the child death-rate goes down about 1 per cent. for every rise of 1 per cent. in the infantile death-rate.

As we have seen, the correlations are not as high in the case of the females as in that of the males. This is probably, to some extent, due (i) to the ages of the women being less reliable than the ages of the men, and this would affect *e*, and (ii) to the lower infantile death-rate in women, the selection being very likely pushed to a higher age; the mere difficulties of birth are greater in the case of the boy and selection may thus be more immediate and stringent in his case. The lower values of the correlations make the agreements between observed and calculated values less close, still there is nothing much to complain of here:—

Period.	Observed.	Calculated.	Δ.
1838—1854	132·60	134·22	+1·62
1871—1880	124·70	119·86	−4·84
1881—1890	98·55	103·55	+5·00
1891—1900	89·93	87·98	−1·85

The remarkable feature of these English life-tables has been the falling child death-rate accompanying the rising infantile death-rate, a phenomenon which should have led those who assert that a high infantile death-rate implies a high death-rate in the next four years of life to pause. It is noteworthy that the Registrar-General has drawn up life-tables in six cases for "selected material," *i.e.* for "selected healthy districts."* These tables give the following results:—

Period.	Males.			Females.		
	i.	*c.*	*e.*	*i.*	*c.*	*e.*
1849—1853 ...	112·80	82·40	53·94	92·64	79·13	53·48
1881—1890 ...	119·15	61·30	56·37	93·50	56·62	57·31
1891—1900 ...	121·50	51·31	57·52	95·08	48·32	58·80

Without venturing to work out anything on the basis of two sets of three tables, we can yet recognise from the crude numbers that precisely the same phenomena occur even in the "healthy districts," *i.e.* improving environment is related to an increasing not a decreasing infantile mortality and while the child mortality decreases with better conditions, it is highly correlated in a negative sense with infantile mortality, a correlation which will not be reduced but emphasized when correction is made for the secular change in environment.*

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* 'Supplement to the 65th Annual Report,' Part I, pp. lviii—cxi, 1907.

It is not my purpose to enter into a discussion of the rise in infantile mortality which has gone on in this country since the fall in the birth-rate started, but I think enough has been done to indicate that when allowance is made for environment, a heavy infantile death-rate indicates a reduced child death-rate. Further, so far from the conclusion that "a high infant death-rate in a given community implies in general a high death-rate in the next four years of life" being true, it is quite incorrect for 14 of the most important English life-tables. These tables support parallel evidence of other kinds, that the Darwinian theory has application to civilised man, and that a heavy death-rate does mean the elimination of the weaklings. To recognise this as a scientific law which controls the evolution of man as of other species is one thing; to assert that the men of science who accept it desire themselves or through the State to play Herod to our modern infants is another thing and a pernicious thing. It is the duty of science to discover what is happening in the first place, and there appears to me a fair amount of evidence now to show that Darwinism does apply to man, and that, for a constant environment, the higher the infantile death-rate, the more resistant will be the surviving child population.

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* Because the correlation between c and e is so nearly perfect that the term $\sqrt{(1-r_{ce}^2)}$ in the denominator of ${}_e r_{ci}$ dominates the relationship.

The Morphology of Trypanosoma simiæ, *sp. nov.*

By Colonel Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey, and A. E. Hamerton, D.S.O., R.A.M.C.; Dr. J. B. Davey, Nyasaland Medical Staff; and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1911–12.)

(Received August 3, 1912.)

[Plate 13.]

Introduction.

This species of trypanosome, which does not seem to have been described before, is remarkable in that it attacks only such widely different animals as the monkey and the goat. Oxen, baboons, dogs, guinea-pigs, and white rats appear to be immune. The rapidity with which it kills monkeys is very striking. In a series of 19 the average duration of life after the trypanosomes were first seen in the blood was only 2·9 days. Its action on animals, its reservoir, its carrier, and cultivation, have not been fully worked out, and will form the subject of a future paper. In regard to its carrier, it may be stated that in this district it is *Glossina morsitans*, and that scarcely a single cage of flies is brought to Kasu Hill from the neighbouring "fly-country" but is found to be infected with this trypanosome.

A. *Living, Unstained.*

Trypanosoma simiæ shows active translatory movements when alive: some individuals pass completely across the field of the microscope. Apparently the usual mode of progression is flagellum first, but occasionally an individual can be seen to move a short distance in the opposite direction.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured, as previously described in the 'Proceedings.'*

Length.—The following table gives the length of this trypanosome as found in the monkey and the goat, 500 trypanosomes in all.

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of *Trypanosoma simiæ*.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Feb. 12	117	Goat	Osmic acid	Giemsa	17·2	18·0	15·0
„ 15	117	„	„	„	16·8	18·0	15·0
„ 22	109	„	„	„	16·7	18·0	15·0
Mar. 11	117	„	„	„	16·5	19·0	14·0
„ 18	247	„	„	„	18·2	21·0	15·0
June 13	620	„	„	„	18·4	21·0	17·0
„ 13	620	„	„	„	17·4	20·0	15·0
Jan. 29	20	Monkey	„	„	17·6	19·0	16·0
Feb. 1	20	„	„	„	17·5	20·0	15·0
„ 6	54	„	„	„	18·9	22·0	16·0
„ 6	55	„	„	„	17·5	20·0	15·0
„ 8	54	„	„	„	18·5	20·0	16·0
„ 12	49	„	„	„	18·4	21·0	15·0
„ 12	54	„	„	„	18·7	24·0	17·0
„ 12	59	„	„	„	17·5	19·0	15·0
„ 23	58	„	„	„	16·0	18·0	14·0
„ 27	58	„	„	„	17·4	19·0	16·0
Mar. 18	286	„	„	„	16·1	18·0	14·0
Apr. 4	58	„	„	„	18·6	20·0	15·0
„ 8	286	„	„	„	17·2	20·0	15·0
„ 18	404	„	„	„	19·8	22·0	16·0
„ 18	448	„	„	„	20·4	23·0	18·0
„ 23	405	„	„	„	19·0	21·0	17·0
„ 25	449	„	„	„	18·6	21·0	16·0
„ 29	448	„	„	„	18·9	21·0	16·0
					17·5	24·0	14·0

The average length of *T. simiæ* in the monkey and goat, taken from Table I, is as follows:—

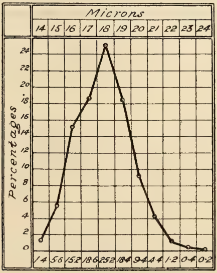
Table II.—Average Length of *T. simiæ*.

Species of animal.	No. of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Monkey	360	18·1	24·0	14·0
Goat.....................	140	17·2	21·0	14·0

Table III.—Distribution in respect to Length of 500 Individuals of *T. simiæ*.

Animal.	In microns.											Average length.
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	
Goat	—	1	5	5	9	—	—	—	—	—	—	17·2
"	—	3	8	7	2	—	—	—	—	—	—	16·8
"	1	2	5	8	2	2	—	—	—	—	—	16·7
"	2	1	10	1	3	3	—	—	—	—	—	16·5
"	—	1	2	3	5	5	2	2	—	—	—	18·2
"	—	—	—	3	9	6	1	1	—	—	—	18·4
"	—	2	2	7	4	4	1	—	—	—	—	17·4
Monkey.........	—	—	4	7	8	1	—	—	—	—	—	17·6
"	—	1	2	7	6	3	1	—	—	—	—	17·5
"	—	—	1	3	6	2	4	3	1	—	—	18·9
"	—	2	3	8	3	3	1	—	—	—	—	17·5
"	—	—	1	3	5	6	5	—	—	—	—	18·5
"	—	2	1	2	5	7	2	1	—	—	—	18·4
"	—	—	—	3	8	4	4	—	—	—	1	18·7
"	—	1	6	4	5	4	—	—	—	—	—	17·5
"	2	5	7	2	4	—	—	—	—	—	—	16·0
"	—	—	4	6	8	2	—	—	—	—	—	17·4
"	2	4	6	5	3	—	—	—	—	—	—	16·1
"	—	1	1	—	5	9	4	—	—	—	—	18·6
"	—	2	5	4	6	2	1	—	—	—	—	17·2
"	—	—	1	1	2	4	4	5	3	—	—	19·8
"	—	—	—	—	2	3	6	5	2	2	—	20·4
"	—	—	—	1	5	8	5	1	—	—	—	19·0
"	—	—	1	3	5	7	2	2	—	—	—	18·6
"	—	—	1	—	6	7	4	2	—	—	—	18·9
Total	7	28	76	93	126	92	47	22	6	2	1	
Percentages ...	1·4	5·6	15·2	18·6	25·2	18·4	9·4	4·4	1·2	0·4	0·2	

CHART 1.—Chart giving Curve representing the Distribution, by Percentages, in respect to Length of 500 Individuals of *T. simiæ*.



From this curve it will be seen that *T. simiæ* is a monomorphic species, varying from 14 to 24 microns in length, the greatest number of individuals (25·2 per cent.) being 18 microns long.

Breadth.—Measured across the broadest part *T. simiæ* averages 1·75 microns in breadth (maximum 2·75, minimum 1).

Shape.—These trypanosomes are monomorphic and, as a rule, fairly uniform in shape. The body is elongated, markedly undulating, and frequently extends in a straight line (Plate 13, fig. 2). The posterior extremity is bluntly pointed or rounded, and there is frequently the appearance of a vacuole at the extreme end, as shown in figs. 6, 9, 11, 16, 17, and 18. The anterior extremity is pointed.

Contents of Cell.—Clear, homogeneous, and free from granules.

Nucleus.—Oval, and situated about the middle of the body.

Micronucleus.—Small and round, situated almost invariably about 1½ microns from the posterior extremity. A peculiarity is that it is almost always placed at the edge of the trypanosome, from which it seems to protrude, or to be on the point of falling out. This is so marked that in the laboratory this trypanosome became known as the "glad eye," from the well-known play of this name.

Undulating membrane.—Well developed and thrown into bold undulations, herein differing from *T. vivax* and *T. uniforme.*

Flagellum.—It is difficult to say whether this species has a free flagellum or not. By careful staining and good illumination it would seem in most cases as if the undulating membrane extended to the tip of the flagellum. In most preparations, however, the last two or three microns of the flagellum often appear to be free. This is shown in Plate 13.

Division Forms.—In the monkey, in which these trypanosomes swarm in enormous numbers, masses of them can be seen, sometimes filling up the whole field of the microscope. It would seem as if multiplication took place so rapidly that the individual trypanosomes had not time to disengage themselves. A small part of such a mass is represented in fig. 1 in the text.

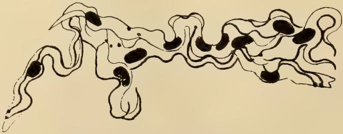
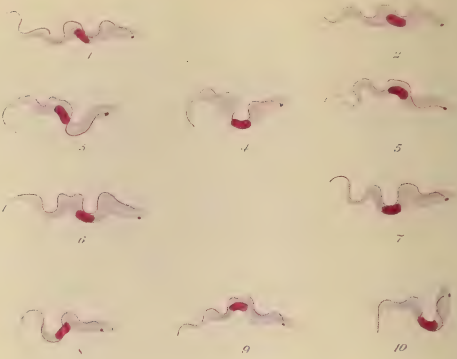
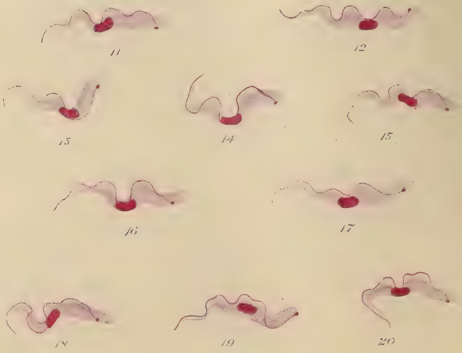


Fig. 1.


1
2
3
4
5
6
7
8
9
10

Monkey.


11
12
13
14
15
16
17
18
19
20

Goat.

M. E. Bruce, del. Grout sc. & imp.

Trypanosoma simiæ, sp. nov.

In addition to this, numerous division forms are seen, often four or five in a field, in which the trypanosomes appear to slip past one another until they are only joined by their non-flagellar ends, as shown in fig. 2.

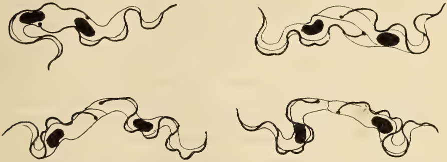


FIG. 2.

CONCLUSIONS.

1. *T. simiæ*, sp. nov., is a well-defined species, easily separated by its morphology alone from the other trypanosomes which have been described as causing disease among domestic animals.

2. It sets up a chronic disease in goats, but is chiefly remarkable for its rapidly fatal action on monkeys.

3. In Nyasaland it is carried by *G. morsitans*, and in this district—Central Angoniland—this tsetse-fly is found to be heavily infected with this trypanosome.

DESCRIPTION OF PLATE.

Trypanosoma simiæ, sp. nov.—Elongated, narrow, undulating body; posterior extremity bluntly pointed or rounded; anterior extremity pointed; nucleus oval; micronucleus small, round, situated about 1·5 microns from posterior extremity, placed laterally, protuberant; undulating membrane marked, thrown into bold folds; flagellum frequently not projecting beyond undulating membrane, sometimes 1 or 2 microns of the extremity apparently free.

Figs. 1—10, *T. simiæ* from the monkey; figs. 11—20 from the goat. Stained Giemsa. × 2000.

The Cultivation of Trypanosoma rhodesiense, *Stephens and Fantham.*

By H. Bayon, M.D., Genoa and Wurzburg, Beit Memorial Research Fellow, Lister Institute.

(Communicated by Sir Ronald Ross, K.C.B., F.R.S. Received June 20, 1912.)

Employing a strain of *Trypanosoma rhodesiense* kindly supplied to me by Sir Ronald Ross, K.C.B., F.R.S., I have carried out some cultural experiments. The following media were found to be successful. (1) Clegg's *Amœba* agar to which is added twice its amount of rabbits' blood which has been frozen and thawed rapidly so as to cause the hæmoglobin to diffuse into the serum; and (2) the following formula:—agar 15 grm., glucose 10 grm., water 1000 grm., and twice the volume of rabbits' blood added.

The trypanosomes were taken from sub-inoculated rats on the third day of infection. Five drops of blood were placed on the agar, which is a diffluent mass resting at the bottom of the culture tube. These were incubated at 22° to 25° C. The tubes were examined after five days and then every two days, usually, up to the 30th day. It was found that if the culture was successful a multiplication of the trypanosomes occurred, which could be seen actually taking place under the microscope. The trypanosomes were easily recognised as *T. rhodesiense* because of the size and position of the kinetonucleus, the length and breadth of the posterior end, and the position of the trophonucleus. This trypanosome was differentiated from other easily culturable trypanosomes, as for example *T. lewisi*, by inoculation into rats. 0·5 c.c. of the diffluent culture injected intra-peritoneally caused *T. rhodesiense* to appear in the blood 10 days after inoculation and the rat died in a further five days. From these rats the trypanosomes were recovered and again cultivated.

The forms of trypanosomes as commonly seen in these cultures are long and slender with a definite flagellum. They move sinuously, with the flagellar end first. The nucleus is longer and narrower than that seen in the blood, and stains with Giemsa's method a light purple. Volutin-granules are frequently present. The kinetonucleus can be seen separated from the blepharoplast and its usual position is somewhat to the side of the trypanosome. These forms remain actively motile up to the 21st day of cultivation. They are virulent up to the eighth day of culture in the quantity inoculated as mentioned above. In certain cases rosette forms are imitated by the trypanosomes clumping together; however, a little pressure on the cover-glass causes them to separate and to swim away individually. True brood-forms have also been observed and distinguished from those clumped. In some culture tubes, rounded (involution) forms appear in great numbers on the third day to the fifth day, and the virulence and properties of these forms are now being separately studied.

The percentage of successes varies very much indeed—from 1 tube in 25 to 6 in 6. Numerous other media have been used, giving invariably negative results by the fifth day, including those consisting of frozen and thawed rabbits' blood serum only.

Further Observations on the Recovery of Trypanosoma gambiense *from* Tragelaphus spekei *on the Islands of Lake Victoria Nyanza*

By Dr. H. L. Duke.

(Communicated by Sir John Rose Bradford, K.C.M.G., Sec. R.S. Received July 17, 1912.)

In a paper* dealing with the recovery of a trypanosome from wild antelope on Damba Island, Lake Victoria Nyanza, the diagnosis is discussed at some length with a view to the exclusion of *T. brucei.*

Fifteen subinoculation experiments were considered and the conclusion was arrived at that the available evidence pointed to the organism being *T. gambiense.* After a lapse of some four months this opinion has received considerable support from a more prolonged investigation of the animal reactions of the trypanosome. In the following table many of the original subinoculations are followed to their conclusion on the death of the animal, and several other experiments have been added to the list.

It will be seen that the evidence supplied by the rat experiments is strongly in favour of *T. gambiense.*

In the six experiments conducted with rats the average duration of the disease is 61 days. This constitutes very strong evidence against *T. brucei.* In guinea-pigs (three experiments) the average duration is 67 days: in five completed monkey experiments, 147 days.

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* "Antelope and their Relation to Trypanosomiasis," 'Roy. Soc. Proc.,' B, vol. 85, p. 156.

Animal.	Expt. No.	Source of virus.	Incubation in days.	Duration of disease in days.	Remarks.
Monkey ...	401	Situtunga 402—3	8	144	
„ ...	511	„ 509—10	12—13	144	
„ ...	504	Flies from 401	?	195	
„ ...	525	Goat 512	10	207	
„ ...	543	Calf 478	10	—	Still alive after 192 days. Active; shows no marked symptoms. Slight wasting.
„ ...	575	Goat 579	6	46	This monkey was an old one, and somewhat emaciated at commencement of experiment.
White rat...	477	Monkey 401	5	83	
„ ...	571	„ 401	5	44	A young rat.
„ ...	639	„ 511	7	51	
„ ...	640	„ 511	7	52	
„ ...	646	„ 401	7	62	
„ ...	647	„ 401	7	74	
Guinea-pig	458	„ 401	10	61	
„	572	Rat 477	20	62	
„	638	Monkey 511	14	80	
Goat	512	Situtunga 509—10	15 (?)	—	Has suffered from abscess on leg, from which it recovered rapidly. Apparently in excellent health after 220 days.
„	579	Monkey 401	?	—	Presence of trypanosomes proved by subinoculation into monkey. Never seen in peripheral blood. Killed after 224 days, showing great weakness; some emaciation.
Dog	573	„ 401	12	96	Extremely emaciated; some opacity of cornea. Killed when at point of death.
„	574	Goat 512	14	43	In very poor condition at the time of inoculation and throughout the experiment.
Calf	478	Monkey 401	?	—	Proved by subinoculation into monkey. Trypanosomes seen only once in peripheral blood. Apparently in excellent health after 237 days.

For comparison with the reactions of this Damba trypanosome the following experiments may be considered, involving other trypanosomes strains employed at Mpumu.

Two types were selected for experiment, the one represented by various strains recovered from wild Lake-shore flies by feeding them on monkeys; the other an undoubted *T. gambiense*. This last was obtained in Monkey 199 by direct inoculation from a reedbuck in captivity at the laboratory, 15 months after the original infection of this animal with a human strain. It should be noted that in the case of all the strains employed the trypanosomes had more or less recently passed through *Glossina palpalis*. This is also presumably the case with the Damba strain as found originally in the wild antelope. This fact is of considerable importance in that it eliminates all idiosyncrasies acquired during continued maintenance in laboratory animals.

Experimental animal.	Origin of trypanosome strain.	Duration of disease.
		days.
Monkey 113	Kibanga flies direct	85
,, 56	,, ,,	104
,, 563	,, ,,	147
,, 597	Namusenyu flies direct	123
,, 538	Human strain from Monkey 199 through laboratory-bred *G. palpalis*	176
,, 643	,, ,, ,, ,,	111
,, 642	,, ,, ,, ,,	89
White rat 659	Monkey 597 by inoculation	62
,, 660	,, 563 ,,	66
,, 576	,, 113 ,,	57
Guinea-pig 690	Human strain from laboratory antelope	50
,, 1	Monkey 199 through laboratory-bred *G. palpalis*	68
,, 2	,, 113 ,, ,,	81
,, 4	,, 113 ,, ,,	76

The average duration of the disease in rats (three experiments) is 61 days: in four guinea-pigs 68 days: in seven monkeys 119 days.

From a comparison of the above two tables it will be seen that there is a close similarity in the animal reactions of all the trypanosomes employed. In no instance does the disease in rats approach the virulence assumed by *T. brucei*. There is, therefore, no reason to doubt that the trypanosomes recovered from wild Lake-shore flies and from the wild situtunga are indeed *T. gambiense*.

A visit was paid to Sese Islands where Carpenter had recently obtained an infection in a monkey with some 6000 odd flies. *G. palpalis* were found to be very numerous and many situtunga tracks were seen. The natural conditions on the islands visited are, however, apparently very much less favourable to the perpetuation of sleeping sickness by these animals than is the case with Damba. There are tracts of open country, between and behind the forests which fringe most of the Lake shore, where situtunga come out to feed and where there are no tsetse. The antelope do not, therefore, enter a fly area immediately upon leaving the shelter of the forest and papyrus as is nearly always the case on Damba.

Six situtunga were shot during the trip. Their blood was examined in the fresh state, by stained films, and by inoculation into goats and monkeys. By the latter method only was a positive result obtained, *T. vivax* appearing in the goat 13 days after the first inoculation of blood. The two monkeys used in these experiments remained negative to daily examinations for 30 days.

With regard to the appearance of trypanosomes in the goat it must be stated that the animal was sent to Sese direct from Entebbe. I was, therefore, only able to make a few preliminary blood examinations before using it for experiment. In the face of former results with *T. vivax* and the fact that the goat was to all appearance quite healthy, there is every reason to conclude that the trypanosomes came from the situtunga.

Studies on the Reductase of Liver and Kidney.—Part I.

By David Fraser Harris, M.D., D.Sc., F.R.S.E., Professor of Physiology and Histology, and Henry Jermain Maude Creighton, M.A., M.Sc., Dr.Sc., Lecturer on Physical Chemistry, Dalhousie University, Halifax, N.S.

(Communicated by Prof. A. J. Brown, F.R.S. Received May 10,—Read June 27, 1912.)

I. *Introduction.*

The notion has been steadily gaining ground that the reducing powers of animal tissues are due to enzymic action. In March, 1910, one of us* adduced evidence that this so-called "reductase" was active in the press-juice of liver and kidney of sheep, ox, horse, and frog. Soluble Prussian blue, methylene blue, and sodium indigo-disulphonate are all reduced more or less perfectly to leuco-compounds by press juice, whereas by a boiled control they are not.

It seemed very desirable to conduct several lines of investigation arising out of the main contention that the tissues were capable of carrying out reduction-processes because they contained an endo-enzyme, viz., How rapidly does the press juice deteriorate in reducing activity; how does it behave in respect of such comparatively stable but reducible substances as $NaNO_3$; in what way, exactly, does its activity vary with temperature; in what way, if any, is the catalase of the liver related to the reductase? It seemed, in particular, highly desirable that a method capable of following the reduction changes quantitatively should be devised in order to enable us to follow the velocity of any given reaction being studied.

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* Harris, D. Fraser, 'Bio-Chem. Journ.,' 1910, vol. 5, p. 143.

II. *Technique.*

As previous experience had shown the high probability of the enzyme being comparatively insoluble, we decided in some instances to perfuse the liver or kidney, as the case might be, with tap-water rather than with physiological saline. We desired not to have to deal with a saline press juice, because it was hoped that we might be able to employ the method of following changes in electrical conductivity as quantitative in investigating the progress of reduction. Our hopes in this connection were not realised, so that the liver used on March 18, 1912, was perfused with physiological saline.

The preparation of the juice on March 27 may be taken as typical. We were supplied with the half of an ox liver from an animal which had just been killed, and this we perfused with tap-water heated to 40° C. until the emergent water was bloodless. The liver was then cut into large pieces, and a good deal of the water allowed to flow out of it. The pieces were then cut up into much smaller portions and forced into the juice-press, in which they were crushed under very considerable pressure. A fawn coloured, viscid liquid dripped out and was received under toluene. This juice was subsequently filtered through cheese-cloth to free it of connective tissue and the debris of blood-vessels. Juices were similarly prepared on January 16 from a frozen ox-kidney, on February 5 from perfectly fresh pig's liver, and on March 18 from pig's liver.

III. *Experimental.*

(a) *Observations with Methæmoglobin.*—We were very anxious to make observations on the action of the juice on hæmoglobin. It was not possible at the time of our experiments to get a sufficient quantity of fresh mammalian hæmoglobin, so that we used dried "hæmoglobin scales, soluble, Merck." On dissolving some of these dried scales, and filtering the solution, we found that the pigment gave the spectrum of methæmoglobin, that is, had the band in the red along with the two bands of oxyhæmoglobin. The solution was stable, and was unaltered by bubbling air (freed from CO_2) through it for four hours; the colour was brown, and never became crimson, so that it was still methæmoglobin. Two equal volumes of the brown solution were now taken, and to one of them 6 c.c. of ox-liver juice, two days old, were added, and to the other 6 c.c. of the same juice heated for 10 minutes at 100° C. The juice and the methæmoglobin were shaken together and placed in a thermostat at 40° C. In less than 10 minutes the unboiled tube was of a bright pink colour, the boiled control being of the original rusty brown. On filtering off, the clear, pink liquid from the opaque juice, and examining it with the spectroscope, the two bands of oxyhæmoglobin were very distinct, while the liquid filtered from the control was still methæmoglobin. We concluded that we had here reduction effected by the active juice, while the heat-inactivated juice was quite unable to effect any reduction. We had, in fact, an illustration of the action thus described by the late Dr. Gamgee in writing of methæmoglobin.* "The action of reducing agents reveals . . . . that the molecule of loose oxygen of hæmoglobin is still present in blood which has been acted upon by nitrates for, in the absence of all traces of oxygen, reducing agents first of all and instantaneously liberate oxyhæmoglobin, which is only afterwards reduced." In other words, the liver press juice acted towards a solution of methæmoglobin exactly as the "reducing agents" to which Dr. Gamgee alluded in the paragraph just quoted.

(b) *The Action of Press Juice on Hydrogen Peroxide.*—It was found that hydrogen peroxide was rapidly decomposed by small quantities of press juice, a few drops of the juice causing relatively large quantities of 3-per-cent. hydrogen peroxide to froth violently at room temperature.

A few preliminary experiments on the rate of decomposition of hydrogen peroxide by press juice were carried out. In these experiments the solutions of the peroxide and the press juice were separately brought to the required temperature before mixing. The mixture was then placed in a thermostat at this temperature, and kept agitated by a mechanical device. From time to time definite amounts of the mixture were removed with a pipette, acidified with sulphuric acid, and the concentration of the hydrogen peroxide estimated by titrating with standard potassium permanganate solution. As potassium permanganate is slowly reduced by organic matter, the titrations were made very rapidly, so as to lessen any error due to the presence of the proteins contained in the juice. The amount of organic matter contained in the mixture pipetted off for titration was always small, and, as very small quantities of press juice were used, was practically negligible.

A mixture containing 190 c.c. of 0·0950 molar hydrogen peroxide and 5 c.c. of press juice (one week old) was prepared and kept at 25° C. It was found that at the end of five minutes, 97·2 per cent. of the hydrogen peroxide had decomposed, and at the end of 15 minutes 97·7 per cent. Another experiment was carried out at 25° C. with a mixture containing 190 c.c. of 0·0950 molar hydrogen peroxide solution, 4 c.c. of distilled water, and 1 c.c. of a press juice solution made by diluting one volume juice with nine volumes of water (*i.e.* the concentration of the press juice in the mixture was 1/50 that in the previous experiment). The following table shows the change in hydrogen peroxide concentration with time :—

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* Gamgee, 'Schäfer's Text-book of Physiology,' 1908, vol. 1, p. 245.

Time.	0·0332 N. $KMnO_4$ required by 10 c.c. of the reaction mixture.	Amount of hydrogen peroxide decomposed.
mins.	c.c.	per cent.
0	55·72	0·0
5	23·91	57·1
10	12·52	77·6
15	6·94	87·6
20	4·32	92·3
25	2·70	95·2
35	1·33	97·6
45	0·67	98·8
70	0·25	99·5

With boiled press juice no decomposition of hydrogen peroxide occurred.

In order to determine the action of proteins on hydrogen peroxide the rate of decomposition of hydrogen peroxide, at 25° C., in a mixture containing 190 c.c. of 0·0950 molar peroxide solution and 5 c.c. of 15-per-cent. white-of-egg solution was determined. The following table gives results obtained:—

Time.	0·0332 N. $KMnO_4$ required by 10 c.c. of the reaction mixture.	Amount of hydrogen peroxide decomposed.
mins.	c.c.	per cent.
0	55·72	0·0
5	54·72	1·8
10	53·84	3·4
15	53·84	3·4
30	53·80	3·4
60	53·71	3·6

It will be seen from the results given in the foregoing tables that the decomposition of hydrogen peroxide by the press juice of liver cannot be attributed to the organic matter contained in the juice; for in the first experiment the amount of organic matter is extremely small, and in the second, where the amount of organic matter is relatively large, only a small amount of the hydrogen peroxide was found to have been decomposed, even at the end of an hour. Since it has been observed by Spitzer* that the liver is rich in catalase, it is most probable that the decomposition of hydrogen peroxide by liver press juice is due to the presence of this enzyme. If, however, the press juice contains a reducing endo-enzyme, as we suppose, it is not improbable that the decomposition of the hydrogen peroxide may be due to the combined action of the catalase and the reductase.

(c) *Experiments with Soluble Prussian Blue.*—It has already been shown by one of us* that soluble Prussian blue is readily reduced to a leuco-compound by small quantities of press juice from liver and kidney. This action has been confirmed and further studied in the present investigation.

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* Spitzer, 'Pflüger's Archiv,' 1897, vol. 67.

Some experiments carried out with the press juice prepared on March 27 are described below.

Three cubic centimetres of the absolutely fresh press juice were shaken up in a test-tube with 10 c.c. of 0·05-per-cent. solution of soluble Prussian blue at room temperature. The blue colour began to disappear immediately, and in less than a minute, after passing through light blue, light green, and greenish-grey, the mixture became pure light grey in colour. When the same volume of boiled press juice was used, no decrease in the intensity of the blue colour of the solution was observed at the end of several hours. The reducing activity of the juice was found to diminish rapidly with time. With a mixture containing 3 c.c. of the press juice 24 hours old, and 10 c.c. of 0·05-per-cent. soluble Prussian blue solution, it was found that 10 minutes elapsed before its colour became greenish-grey, and two hours before it became completely grey; and when the juice was four days old it was necessary to allow the mixture to stand under toluene for seven or eight hours in order to obtain a greenish-grey colour. On adding a few drops of hydrogen peroxide to some of the light grey mixture, the blue colour was immediately restored. The blue colour of the mixture was also slowly re-established by allowing it to stand exposed to the air. This re-blueing commenced at the surface of the mixture and slowly progressed downwards. If the decolorised mixture was spread out in a thin layer on a watch glass, the colour quickly returned. It has been found on allowing the Prussian blue press juice mixtures to stand, that the fawn-coloured protein matter of the juice usually settled to the bottom of the test-tube, and that a white grey substance remained suspended above it. Some of this grey suspension was pipetted off. It was found that it rapidly turned bluish-green on exposure to the air or on being treated with hydrogen peroxide. This blueing of the whitish grey suspended material supports the suggestion of Harris and Irvine† that potassium ferrous ferrocyanide is formed when soluble Prussian blue is acted upon by liver press juice. As has been already mentioned, the colour of soluble Prussian blue, when mixed with small quantities of press juice, gradually fades to a green grey, and after further time has elapsed all trace of green colour disappears, leaving only a pure grey. Further, it has been found that the first colour change takes place more quickly than the second. This phenomenon suggests the formation of intermediate compounds; and, indeed, it would appear that the first stage of the reduction is more readily effected by the reductase of the press juice than the second stage. We intend to investigate the chemical nature of such intermediate compounds.

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* Harris, D. Fraser, *loc. cit.*

† Harris, D. Fraser, and J. C. Irvine, 'Bio-Chem. Journ.,' 1906, vol. 1, p. 357.

A number of experiments was carried out with a view to determining the relation of the reducing activity of the press juice to temperature. In these experiments, the results of which are given below, 3 c.c. of a 24-hour-old juice were mixed with 10 c.c. of 0·05-per-cent. solution of soluble Prussian blue. Both the soluble Prussian blue solution and the press juice were brought to the temperature of the experiment before mixing.

Temperature.	Time required for the mixture to become light green-grey.
° C.	mins.
0	40
14	10
25	6
40	2·5 to 3
55	1·25
67	less than 1
72	2

Press juice and a solution of soluble Prussian blue that had been cooled to −2° C. were mixed and placed in a freezing mixture at −14° C. It was found at the end of two hours, although the mixture quickly became solid after immersion in the freezing mixture, that the colour had turned from blue to green. That reductase is not permanently inhibited by a temperature of −14° C. is shown by the fact that when this mixture was melted and warmed, the green colour faded rapidly to light green-grey. It is most probable that, at −14° C., the extremely weak action of the reductase is due not so much to inhibition of the enzyme as to the formation of the solid phase.

Experiments were also carried out at 100° C. The press juice and the solution of soluble Prussian blue were separately brought to this temperature and then mixed. It was invariably observed that the blue colour of the mixture faded to the light green-grey in about one minute. The blue colour could be re-established, however, by shaking up the cooled colourless mixture with a few cubic centimetres of dilute hydrochloric acid. Since it has been shown that the strong activity of a fresh juice is completely destroyed by heating to 100° C. for two or three minutes, it is evident that at this temperature the decolorisation of soluble Prussian blue cannot be due to the action of reductase, but that it must be ascribed to some other cause. In a recent investigation it has been shown* that soluble Prussian blue is decolorised by native and derived proteins, and although this action is inappreciable at room temperature at the end of several hours, and even at 60° C. requires an hour or more for completion, at 100° C. the decolorisation takes place very rapidly. The decolorisation of the blue is due to the formation of a complex with the protein, which is broken down by hydrochloric acid with return of the blue colour. In the light of this investigation, the fading of the colour from mixtures of press juice and soluble Prussian blue at 100° C. must be attributed to some action due to the presence of protein, and not to the action of the reductase.

(d) *The Reduction of Iron Salts by the Press Juice of Liver.*—A few experiments were undertaken to see whether the press juice from liver was capable of bringing about the reduction of ferric salts.

To a dilute solution of ferric chloride, 3 c.c. of two-day-old press juice were added, and the mixture kept at 40° C. for 20 minutes. The protein substances were then filtered off, and a few drops of potassium ferricyanide added to the brown coloured filtrate. On the addition of the potassium ferricyanide, a green-blue precipitate which rapidly turned deep blue was formed, indicating the presence of iron in the ferrous condition. Pure press juice gave no coloration with potassium ferricyanide. In a control experiment, where a ferric chloride solution was warmed with 3 c.c. of press juice which had been boiled, no blue coloration was observed on the addition of potassium ferricyanide.

It will be seen from these experiments that ferric salts are readily reduced to the ferrous condition by the reductase of liver.

(e) *The Reduction of Nitrates.*—In a recent investigation, Kastle and Elvove† have shown that various inorganic nitrates are reduced to nitrites by the action of aqueous extracts of certain plants. This suggested the possibility of the reduction of nitrates being brought about by the reductase in liver press juice. Accordingly, a number of experiments was undertaken with this end in view.

In each of two test-tubes, 20 c.c. of two-day-old press juice (prepared March 27) were placed. One of these tubes was placed in boiling water for five minutes. It was then cooled and diluted to its original volume with distilled water. To each tube 20 c.c. of a pure solution of sodium nitrate, containing 5 grm. of the salt, were simultaneously added, and both tubes tightly corked and shaken. The tubes were then placed in a thermostat at 50° C. for an hour and a half, during which time they were frequently shaken. At the end of this time 5 c.c. were pipetted off from each tube, diluted with distilled water, and rapidly filtered from protein material. The clear, light brown filtrates were then decolorised by shaking with powdered animal charcoal, which was subsequently removed by filtration. The colourless filtrates were finally tested for nitrite with Griess' sulphanilic acid reagent, and the amount of nitrite determined colorimetrically. The following are the results obtained :

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* Creighton, H. J. M., 'Trans. Nova Scotia Inst. Sci.,' 1911—1912, vol. 13 (2), p. 61.

† Kastle, J. H., and E. Elvove, 'Amer. Chem. Journ.,' 1904, vol. 31, p. 606.

The nitrite contained in 5 c.c. of the active liquid was found to correspond to 7·65 c.c. of the standard* nitrite solution. Therefore the nitrite contained in the whole solution was equivalent to 1·47186 mgrm. The nitrite contained in the total volume of boiled liquid was less than 0·04 mgrm.

Another experiment similar in every respect to the above, except that the 20 c.c. of the sodium nitrate solution added to each tube contained 2·5 grm. of the salt, gave the following results :

The nitrite contained in 5 c.c. of the active liquid was found to correspond to 5·90 c.c. of the standard nitrite solution. Therefore the nitrite contained in the whole active solution was equivalent to 1·13516 mgrm. The nitrite contained in the total volume of boiled liquid was less than 0·03 mgrm.

From these experiments it will be observed that the amount of reduction brought about by the active press juice is not inconsiderable, while, on the other hand, boiled press juice reduces little or no nitrate. This behaviour affords additional support to the enzymic character of tissue reduction.

As it seemed probable that many important and interesting results regarding the nature of reductase might be obtained by studying the velocity of reduction, we attempted to follow the change in concentration of the soluble Prussian blue solutions by means of electrical conductivity measurements. This method, however, had to be abandoned owing to the extremely small changes in conductivity that were found to occur in the reactions investigated.

Owing to the ease and accuracy with which measurements on the reduction of nitrates can be carried out colorimetrically, we intend, in the immediate future, to employ this reaction for the quantitative study of reductase.

IV. *Summary of Conclusions.*

1. The existence of a catalytic enzyme in the mammalian liver is fully confirmed. The decomposition of hydrogen peroxide is effected by this enzyme and is not due to the presence of proteins or other organic matter in the press juice. We intend to study further the behaviour of this hepatic catalase.

2. We find the existence of a reducing endo-enzyme (reductase) confirmed.

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* 1 c.c. of the standard solution employed contained 0·02405 mgrm. of sodium nitrite.

Methæmoglobin is by press juice reduced at body temperature to hæmoglobin after the manner of non-living, reducing agents. Such a relatively stable compound as sodium nitrate is reduced to nitrite, and ferric chloride to the ferrous condition. These reductions are not due to the proteins of the juices, since a control of boiled juice alters none of the substances hitherto used to demonstrate reduction. We intend to use the nitrate reaction as a basis of a method to follow these reduction changes quantitatively.

3. The probability of the enzymic character of tissue reduction is further confirmed by the effect of certain protoplasmic poisons lately investigated by one of us.* Certain virulent protoplasmic poisons inhibit reductase in virtue of their acidity rather than through their toxicity; this finding is in accord with the well established fact that acidity (concentration of H-ions) inhibits the activity of many enzymes.

4. Though the presence of proteins in press juice is not responsible for such a reduction as that of soluble Prussian blue to the colourless condition, yet the proteins of the juice form with the pigment a colourless chemical or physical compound. This change takes place rapidly at 100° C. and exceedingly slowly at room temperature.

One of us† has shown that such proteins as egg-albumin and gelatine readily form with soluble Prussian blue such compounds at higher temperatures. It would be convenient to allude to these phenomena as the "Creighton effect." We desire to distinguish this fading of pigments through combination with proteins from true vital reduction, and we venture to suggest that the so-called reduction effected by colloids and studied by Heffter‡ may be of the nature of the fading of pigments; it is not the same phenomenon as the reduction which we are studying.

5. We lay a considerable degree of stress on the fact that reductase is able to reduce chemical substances differing very widely in structure, propensities and stability. Not only can it reduce compounds containing oxygen such as methæmoglobin and sodium nitrate, but with equal potency substances which contain no oxygen and are of a relatively stable nature, such as ferric chloride and soluble Prussian blue.

The expenses of this work were met by a grant to one of us (D. F. H.) from the Government Grant Committee, which is hereby gratefully acknowledged.

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* Harris, D. Fraser, 'Bio-Chem. Journ.,' vol. 6, p. 2.

† Creighton, H. J. M., 'Trans. Nova Scotia Inst. Sci.,' 1911—1912, vol. 13 (2), pp. 61—75.

‡ Heffter, A., 'Medizinisch-Naturwissenschaftliches Archiv,' vol. 1, Part 1, p. 81; also 'Archiv f. expr. Path. und Pharm.—Festschrift f. O. Schmiedeberg,' 1908, p. 253.

CROONIAN LECTURE: *The Process of Excitation in Nerve and Muscle.*

By KEITH LUCAS.

(Communicated by Sir John Bradford, Sec. R.S. Received June 6,—Lecture delivered June 6, 1912.)

To physiologists working half a century ago it must have seemed that there was scarcely a problem of their science more hopeful of solution than that of the physico-chemical nature of the nervous impulse. Helmholtz had recently measured the rate at which the impulse is propagated along a nerve. Du Bois Reymond's researches had placed the study of animal electricity on a scientific basis, and there was just commencing that work of Bernstein which coupled the two phenomena by mapping out the time-relations of the electric change which sweeps along an active nerve, and showing how this change keeps time with the nervous impulse.

But the problem is still unsolved; indeed, the progress towards solution was for a long time of such a nature that physiologists were able 10 years ago to write such words as these: "After decades of work by so many distinguished investigators we are as far as ever from the understanding of the intimate nature of nervous excitation,"*; or, again: "In recent times voices have been heard belittling the scientific value of electrophysiology†"; or, in the more picturesque words of v. Uexkull‡: "For whole decades the frog's leg has been investigated without being crippled by the shrewdest heads and the most talented experimenters."

I do not come before you to-day with any new solution of this problem. Nor shall I even attempt to criticise those many solutions which physiologists have offered from time to time. My aim will be more elementary than that; it will be to enquire into that fundamental analysis of the biological phenomena concerned, which must precede any formulation of physico-chemical hypotheses, and to see how far that analysis can guide us towards the building of our hypothesis on a sure foundation. To each investigator of the phenomena of the excitable tissues there must stand out pre-eminently some aspect of past work which will seem to account for the slow measure of progress. By some of those writers whom I have quoted the cause of failure was located in the too artificial handling of the problem as one of physics rather than of biology. As v. Uexkull* remarks: "They had reckoned without their host, and the host's name was Life." To others, the mistake has seemed to lie rather in too exclusive a consideration of the physical side of the phenomena and a consequent neglect of the chemical factors involved.† The aspect of the problem which has appealed to me is that in nerve and muscle we have to deal with a complex sequence of phenomena, some intimately, and some only more remotely concerned with the actual propagated disturbance which is the basis of the nervous impulse. The phenomena which precede, accompany, and follow the propagated disturbance form the chief data on which we have to rely in formulating our hypothesis as to the physico-chemical nature of that disturbance, and we can build on a sure basis only when we have a rigid experimental knowledge of the way in which these phenomena are inter-related. It is perhaps the surprising feature of the past history of this investigation that out of so rich a body of experimental work on the individual phenomena we can gather so small a number of established propositions as to their mutual dependence and inter-relation. And yet it is in this particular problem of the nature of the propagated disturbance, if in any, that the question of inter-relation must play a fundamental part. The disturbance is an unknown change which sweeps along the excitable tissue, and we can come to grips with it only through the conditions by which it is initiated and the measurable effects which it leaves behind.

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* Biedermann, 'Ergeb. d. Physiol.,' 1903, vol. 2, Part 2, p. 132.

† Loeb, 'Arch. f. d. ges. Physiol.,' 1902, vol. 91, p. 248.

‡ v. Uexkull, 'Leitfaden in d. Stud. d. Exp. Biologie,' Wiesbaden, 1905, p. 5.

Let us turn to this question of analysis and consider first what is the complex of phenomena with which we have to deal. For the purpose of this enquiry I shall choose the artificial case of an isolated motor nerve with its attached muscle, the nerve being stimulated at one point by the passage of a brief electric current along a small part of its length. I choose here just those artificial conditions and that artificial stimulus to which some investigators have objected, and I shall hope presently to justify the choice. The principal and most obvious phenomena consequent upon the application of the stimulus are these. A change of unknown nature travels along the nerve in both directions from the seat of stimulation; it is this change which I shall speak of as the propagated disturbance and not by the more specialised name of the nervous impulse, because there is evidence that a change of similar nature travels along muscle-fibres also. We are made aware that the propagated disturbance has travelled along the nerve by the changes which it effects in the muscle on its arrival there, but there are two measurable changes in the nerve itself which also follow the application of the stimulus; these are a redistribution of electric potential in the nerve (the electric response) and the failure of a second stimulus to produce like effects for a period of a few thousandths of a second (the refractory state). On the arrival of the propagated disturbance at the muscle all the phenomena which occurred in the nerve are repeated there, and, in addition, the muscle undergoes a mechanical contraction and heat is liberated. We have to enquire how each of these phenomena is related to the propagated disturbance.

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* *Loc. cit.*

† Höber, 'Zeitschr. f. allg. Physiol.,' 1910, vol. 10, Sammelreferat, p. 173.

A. *The Local Excitatory Process.*

Consider, then, the first link in this chain, the setting up of the propagated disturbance by the electric current. Is this all, or does the current itself bring about some intermediate change which then provides the necessary condition for starting the propagated disturbance? The question is of fundamental importance to our analysis, because if such a preliminary process is involved we may hope, from a study of the conditions to which an adequate stimulating current must conform, to learn its physico-chemical nature; we shall then be in possession of the conditions within a tissue by which the propagated disturbance is started, and shall have made one step towards the identification of that disturbance.

The classical method of dealing with this problem has been to show that various substances, such as carbon dioxide, ether, and alcohol, which gradually suspend the functional activity of nerve, alter at different rates the ease with which a propagated disturbance already started can travel along the nerve, and the ease with which such a disturbance can be initiated by an external stimulus. In the usual terms, there is a difference in the rates of abolition of "conductivity" and "excitability." The method, used first by Grünhagen,* and later by many observers who followed him, is to treat with any of the narcotics only a part of the length of the motor nerve innervating a muscle, leaving the portion more remote from the muscle unaffected. From time to time during the progressive action of the narcotic determinations are made of the strength of stimulus required to cause a contraction of the muscle both when the stimulus falls actually within the narcotised region of the nerve, and when it falls on the normal part beyond. If the narcotic merely renders the nerve less easily excitable, the stimulus applied within the narcotised region will need to be strengthened, whereas that applied to the normal region will need no alteration. If, on the other hand, the narcotic lowers the ability of the nerve to conduct, then the stimulus applied in the normal part may become ineffective, because the propagated disturbance which it sets up has to traverse the narcotised region before it can reach the muscle. The results obtained from the application of this method are of two kinds, appearing successively at different stages of narcosis. The earlier stage (first described by Grünhagen)* is that whereas the stimulus applied within the narcotised region has to be strengthened, a stimulus applied in the unaffected region need be no stronger than before. Apparently, then, the propagated disturbance can pass through the narcotised region without difficulty, but is not easily initiated there; the narcotic has altered excitability, but not conductivity. The later stage (first noticed by Szpilman and Luchsinger)† is that the stimulus inside the narcotised region may still make the muscle contract if made very strong, whereas no strength of stimulus applied to the normal part will produce any effect on the muscle whatever. Apparently it has now become impossible for a disturbance to be propagated through the whole length of the narcotised region, though one can still be set up within it.

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* Grünhagen, 'Arch. f. d. ges. Physiol.,' 1872, vol. 6, p. 180.

These facts, which have been verified repeatedly, have been taken as evidence that, in the ordinary physiological terms, "excitability" and "conductivity" can be differently affected, and that, consequently, excitation by an external stimulus must involve a process which is not involved in the propagated disturbance. Is this result to be accepted for our purpose? It is clear from the work of Werigo,‡ Fröhlich§ and Boruttau|| that the later stage of narcosis certainly will not bear any such interpretation. These observers have made it clear that the diminution of a propagated disturbance in a narcotised nerve increases with the length of the narcotised region traversed. The explanation of the second stage of narcosis would therefore seem to be that the propagated disturbance set up in the normal part of the nerve has to traverse the whole length of the narcotised region, and consequently gets wiped out of existence, whereas that set up within the narcotised region has a shorter length of narcotised nerve to traverse, and therefore succeeds. But there still remains the earlier stage of narcosis, in which apparently the excitability is altered, while the conductivity is still normal. Here, again, it is probable that the apparent dissociation of the two processes is illusory. Wedensky¶ found that, though a single disturbance appears to get through the narcotised region without diminution at a time when excitability is already obviously altered, yet the attempt to send a rapid succession of disturbances along a nerve in this

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* *Loc. cit.*

† 'Arch. f. d. ges. Physiol.,' 1881, vol. 24, p. 347.

‡ Werigo, 'Arch. f. d. ges. Physiol.,' 1899, vol. 76, p. 552.

§ Fröhlich, 'Zeitschr. f. allg. Physiol.,' vol. 3, p. 148.

|| Boruttau and Fröhlich, 'Zeitschr. f. allg. Physiol.,' vol. 4, p. 153.

¶ Wedensky, 'Arch. f. d. ges. Physiol.,' 1900, vol. 82, p. 134.

condition reveals the fact that the conduction process is really no longer normal. Boruttau and Fröhlich* also opposed the usual interpretation. They found that the longer the stretch of nerve narcotised the smaller was the fall of excitability observed at the stage of narcosis in which the conduction process first began to fail. It would seem probable from this result that if we could use a sufficiently long nerve we should find that even the smallest perceptible change of excitability brought with it a measurable change in conductivity, and that our failure to recognise the latter in the ordinary experiment lies merely in the fact that the propagated disturbance, though diminished in the course of its propagation through the narcotised region, is so little diminished that it still causes the muscle to contract effectively. This possibility comes before us with even greater force if we take into account the results of some work which Adrian has recently carried out at Cambridge. He has been so kind as to allow me to mention his results, which have not yet been published. He finds evidence that a propagated disturbance passing down a locally narcotised nerve will, if not too far diminished by its passage through the narcotised region, actually recover in size again when it passes out into a normal stretch of nerve beyond. This observation might appear to contradict the experiments of Boruttau,† which showed that a propagated disturbance, after emerging in such a way, was accompanied by an abnormally small electric response, but in reality there is no contradiction, since Boruttau's experiments did not exclude the possibility that the reduced response was due to some fibres having failed to propagate any disturbance at all. If this result of Adrian's proves to be well founded, it supplies an obvious explanation of the failure of the muscle contraction to indicate small changes in the size of the propagated disturbance transmitted through a narcotised region, for the disturbance, by the time it reaches the muscle, may have recovered its normal size again.

It appears from this evidence that the dissociation of two processes by the analytical method of Grünhagen is in all probability an illusion resting on an inadequate method of determining changes in the size of the propagated disturbance. The evidence is, however, still incomplete. We have no direct experimental proof that the degree of narcosis which first calls for a stronger exciting current within the narcotised region does actually cause a diminution of the propagated disturbance as it passes through that region. Nor will such evidence be obtained until we have an exact experimental method of measuring small changes in that disturbance. As we have seen, the method of measurement in terms of the effect produced by the disturbance after it has emerged into a normal region of the tissue can probably detect only changes so gross that they have led to complete extinction. To measure in terms of the electric response produced in the nerve (a method not infrequently used) is to beg one of the most fundamental questions in the analytical problem with which we are dealing; such a method assumes a knowledge of the relation between propagated disturbance and electric response which we do not possess. It chances, however, that a method which seems capable of detecting extremely small changes in a propagated disturbance has grown out of some experimental work which Adrian and I have recently published.* We found that if two propagated disturbances are sent down a nerve in close succession, the shorter the time-interval by which they are separated the less is the length of slightly impaired nerve which the second disturbance is able to face without extinction.† In fact, the second disturbance is continuously less in intensity the shorter the time-interval separating it from its predecessor. It becomes possible, therefore, so to time two disturbances that the second is only just able to propagate itself successfully along a perfectly normal nerve; such a minimal second disturbance will at once be extinguished if the "conductivity" of the nerve is in the least degree impaired.

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* Boruttau and Fröhlich, *loc. cit.*

† Boruttau, 'Arch. f. d. ges. Physiol.,' vol. 65, p. 7.

This method is now being applied in the Cambridge Laboratory to test whether during the earliest stages of narcosis the slight changes of excitability usually observed to precede any gross change of conductivity are in reality accompanied by a small but measurable diminution of the propagated disturbance in the course of its travel. When the investigation is complete we hope to have an answer to this long-standing riddle of the interpretation of Grünhagen's experiment.

As a means of analysis then this method of dissimilar alteration has failed us for the present at least. But it does not follow that no differentiation of two processes can be found. I believe, in fact, that along quite other lines evidence has really been available for some years past, merely waiting to be put together in a connected argument.

It is a fact long ago established in physiology that if a short current just too weak to set up a propagated disturbance is applied to a nerve, then for a short time after this current has ceased to flow a second current, otherwise too weak to be effective, will succeed in setting the propagated disturbance in motion. It is clear that some change persists in the nerve after the first current has ceased to flow, and renders effective the otherwise inadequate second stimulus. The important question from the analytical standpoint is whether this persisting change is merely a quantitatively less intense disturbance of the same nature as that which is the basis of propagation, or is an incomplete preliminary process which needs to be of greater intensity before the propagated disturbance can be initiated at all. The evidence on this point seems to establish clearly the fact that the latter alternative is correct; we have to do with a preliminary change. In the first place Adrian and I found that there was no measurable trace of this change being propagated from the seat of the first stimulus.* Also it must be noticed that whereas the persisting after-effect of the inadequate first stimulus is that the tissue can be excited by a weaker stimulus than otherwise, the effect of an adequate stimulus on a nerve is that for a considerable period a second stimulus will either be unable to excite at all, or will need to be considerably strengthened, these being the phenomena of the refractory state which Gotch and Burch† first detected in nerve. It is scarcely probable that the same disturbance should so invert its after-effects by undergoing itself no alteration but a change of intensity. And the evidence in favour of these divergent after-effects belonging to two distinct stages in the setting up of the propagated disturbance becomes far stronger when we examine the effect of temperature change on the persistence of the after-effects of adequate and inadequate stimuli. It was shown by Bazett‡ that a fall of temperature from 18° C. to 8° C. prolongs the time of persistence of the lowered excitability following an adequate stimulus by about 200 per cent. The heightened excitability which follows an inadequate stimulus I found§ to be prolonged under a like change of temperature by less than 40 per cent. The outcome of this evidence I think is clear. We have in the summation of inadequate stimuli proof of the existence of a local change which must be brought about with a certain intensity by the stimulating current before the propagated disturbance can be started. I have called this change the local excitatory process. We shall return presently to examine it more closely, and shall see how a knowledge of its nature is essential to any hypothesis of the propagated disturbance because it is the middleman between that disturbance and the external stimuli which we can control experimentally.

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* Adrian and Keith Lucas, 'Journ. Physiol.,' 1912, vol. 44, p. 68.

† This elementary fact was probably at the root of the observation of Wedensky mentioned above, that a narcotised nerve may appear to be conducting normally when tested with a single stimulus, but may, nevertheless, appear abnormal when tested with periodic stimuli of high frequency.

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* Adrian and Keith Lucas, *loc. cit.*

† Gotch and Burch, 'Journ. Physiol.,' 1899, vol. 24, p. 410.

‡ Bazett, 'Journ. Physiol.,' 1908, vol. 36, p. 426.

§ Keith Lucas, 'Journ. Physiol.,' 1910, vol. 39, p. 469.

B. *The Electric Response.*

We turn now to the second stage of our analysis, the problem how the propagated disturbance is related to the electric response. The starting point of this problem is the work of Bernstein,* which showed that the electric response keeps time with the propagated disturbance in its passage along a nerve or muscle. The fact actually shown was that the time-interval between the culmination of the electromotive force of the electric response at two points on the length of a nerve or muscle agrees with the interval between the passage of the propagated disturbance under those two points, as calculated from the mean rate of travel of the latter. This work established the proposition that the electric response is a normal accompaniment of the propagated disturbance, but does not resolve our doubt completely. We have to enquire further if the association is a necessary one. This point must be fundamental to any hypothesis of the propagated disturbance, because any hypothesis must give an account of the electric response and must know whether to include it as the central feature of propagation or to regard it as a mere accessory.

From time to time physiologists have brought experimental evidence to show that the two phenomena can be dissociated, that there can be an electric response when there is no propagated disturbance or a propagated disturbance with no electrical accompaniment. The most careful experiments of this sort are those of Gotch and Burch.† They observed that when two stimuli separated by a suitable interval of time were applied to a cooled portion of a nerve, the electrometer connected to this part of the nerve showed only a single electric response, whereas in a warmer portion of the nerve more distant from the seat of stimulation the electrometer showed clearly that two separate electric responses were present. They concluded that the second propagated disturbance must have passed along the cooled part of the nerve unaccompanied by such differences of potential as constitute the familiar index of the electrical response, and that the absence of any perceptible electric response does not necessarily imply the absence of the propagated excitatory disturbance. Later‡ Gotch brought evidence that within a short distance of a recent injury a propagated disturbance might pass without any perceptible electric change, a fact in which he saw additional support for the conception that the electrical concomitant of the nervous impulse may under certain conditions fail.

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* Bernstein, 'Untersuchungen über d. Erregungsvorgang im Nerven- u. Muskelsysteme,' Heidelberg, 1871.

† Gotch and Burch, 'Proc. Physiol. Soc.'; 'Journn. Physiol.,' January, 1899, vol. 23, p. xxii; 'Journ. Physiol.,' 1899, vol. 24, p. 422.

‡ Gotch, 'Journ. Physiol.,' 1902, vol. 28, p. 50.

Quite recently Ellison* has described a series of experiments from which he draws the conclusion that an electric response does not necessarily accompany a nervous impulse. A motor nerve is treated with cinchonamine hydrochloride and stimuli are applied within the region so treated. It is found that though the muscle contracts when the nerve is stimulated, no trace of an electric response is shown by a galvanometer connected with a pair of electrodes on the nerve.

Herzen† and Radzikowski‡ attacked the problem in the reverse way, bringing evidence to show that there may be a marked electric response in a nerve along which no propagated disturbance is travelling. Their method was essentially to show that a nerve under the influence of various abnormal conditions would respond to a stimulus with an electric response though no contraction of the muscle resulted. They concluded that the presence of an electric response was no necessary indication that a nervous impulse was present. These experiments were severely criticised by physiologists both as to their technique and as to their interpretation. Wedensky§ and Boruttau|| repeated the experiments and called attention to sources of actual experimental error; it would, however, be superfluous to enter now into the details of that controversy, since recent experimental work has brought to light facts which seem to invalidate the principle upon which the method was founded. Herzen and Radzikowski relied for their proof that no nervous impulse passed along the nerve on the fact that the muscle did not contract. To this inference it was very naturally objected that perhaps the junction between nerve and muscle had shared the abnormal influences which had been brought to bear upon the nerve, and had become impassable to the nervous impulse. Radzikowski¶ was careful to exclude the possibility of any such gross change in the junction between nerve and muscle; but recent work has shown that even in a nerve and muscle recently excised and kept under the best possible artificial conditions the junctional region will be found to offer a certain resistance to propagation. For example, Adrian and I found evidence that in an apparently normal preparation there may often be some nerve-fibres which fail to transmit a single propagated

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* Ellison, 'Proc. Physiol. Soc.,' January, 1911; 'Journ. Physiol.,' 1911, vol. 42, p. i; Journ. Physiol.,' 1911, vol. 43, p. 28.

† Herzen, 'Centralbl. f. Physiol.,' 1899, vol. 13, p. 455.

‡ Radzikowski, 'Arch. f. d. ges. Physiol.,' 1901, vol. 84, p. 57.

§ Wedensky, 'Arch. f. d. ges. Physiol.,' 1900, vol. 82, p. 134.

|| Boruttau, 'Arch. f. d. ges. Physiol.,' 1901, vol. 84, p. 325.

¶ Radzikowski, *loc. cit.*, and 'Centralbl. f. Physiol.,' 1901, vol. 15, p. 273.

disturbance to the muscle.* Such facts as these suggest how dangerous it is to argue from the failure of the muscular contraction to the absence of a propagated disturbance in the nerve. The danger is especially great when, as in the experiments of Herzen and Radzikowski, we have to deal with nerves under abnormal influences and likely therefore to transmit disturbances abnormal either in their time-relations or in their magnitude. Until we have some index of the presence and absence of the nervous impulse more reliable than its success in passing the junctional region and provoking the muscle to contraction, we cannot accept as proved the occurrence of an electric response in nerve unaccompanied by a nervous impulse.

We must turn, therefore, to the evidence brought in favour of the converse proposition, that the propagated disturbance may be present with no electrical accompaniment. I fear that the experiments of Ellison, which I have mentioned, give us very little help in this matter, at least in the present state of the enquiry. Dittler and Satake† have repeated them within the present year and fail to confirm the results which Ellison obtained. They find in fact that cinchonamine hydrochloride, like other drugs which suspend the activities of nerve, abolishes simultaneously the nervous impulse and its electrical accompaniment. If one can still be detected so can the other, and as soon as one fails the other shows no trace. They have suggested that Ellison's results rest chiefly on his failure to realise that in a nerve whose conduction process is abnormal the propagated disturbance undergoes a decrement which increases with the length of nerve traversed. Neglecting this factor, he may have so chosen the seat of excitation that the propagated disturbance set up was able to reach the muscle without complete extinction, but failed to cover the longer distance which separated it from the electrodes connected to the galvanometer. The question can hardly yet be thought of as settled, but this much is clear, that without more exact confirmation Ellison's work cannot be taken as evidence that there can be a propagated disturbance unaccompanied by an electric response.

There remain then the experiments of Gotch and Burch. Boruttau‡ repeated those experiments which related to the absence of a second electrical response in a cooled region of nerve and its appearance in a warmer region beyond. He could not satisfy himself that there was ever complete absence of the second electric response in one region unless it was also absent in the other. Gotch,§ after analysis of the photographic records on which he and Burch had founded their original statement, discovered a small trace of a second electric response in the cooled region of the nerve. This response was greatly delayed and might easily have been regarded as a part of the single response. Nevertheless its presence limited the possible inference from the experiment to the statement that an effective propagated disturbance might be accompanied by a scarcely measurable disturbance of electric potential. In the same paper Gotch brought the further evidence that the neighbourhood of a recent injury may be incapable of producing an electric response of sufficient magnitude to affect a sensitive capillary electrometer, though it is a region obviously able to propagate a disturbance, since quite weak stimuli applied to it will provoke a disturbance which is propagated to other parts of the nerve. This observation has not, as far as I am aware, been called in question, and we must admit at least that the nervous impulse may be accompanied by an electric response so small as to be imperceptible by the capillary electrometer.

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* Adrian and Keith Lucas, 'Journ. Physiol.,' 1912, vol. 44, p. 90.

† Dittler and Satake, 'Arch. f. d. ges. Physiol.,' 1912, vol. 144, p. 229.

‡ Boruttau, 'Arch. f. d. ges. Physiol.,' 1901, vol. 84, p. 402.

§ Gotch, 'Journ. Physiol.,' 1902, vol. 28, p. 52.

Have we here a satisfying proof of want of parallelism between the propagated disturbance and the electric response? In our interpretation of the experiment we must bear in mind the limitations which are imposed upon our measurement of both phenomena, and must find a want of parallelism only if we are clear that whereas the one has undergone very great diminution the other has not. In measuring the electromotive force of the electric response we are at a great disadvantage. It is an old observation, which has lately been brought into prominence,* that the electromotive force which we measure with our electrometers is only an unknown fraction of that actually present in the tissue. We are leading off the fall of potential between two points of that external part of the circuit in a nerve or muscle which is made up of the lymph or other fluid retained between the fibres. What part of the total resistance of the circuit that represents, and consequently what part of the whole potential difference is led off, we do not know. Still more are we unable to obtain a relative measure of the size of a propagated disturbance at any given point in a tissue. Our one method is in practice to measure the consequence of the propagated disturbance—either as muscular contraction or as electric response—after it has emerged from a region where it has been modified. If the experiments of Adrian, of which I have already spoken, prove to be valid, such methods can in reality tell us only whether a disturbance has or has not been extinguished. Consider, for example, such an experiment as that of Gotch and Burch on locally cooled nerve. Within the cooled region the second electric response was scarcely perceptible; in the warmer region beyond it was large. There seems no valid argument against the supposition that the second propagated disturbance was also reduced to minimal intensity within the cooled region (where recovery from the effects of the first disturbance would be slow), and grew up to full dimensions again when it emerged into the warmer stretch of nerve. In fact, we are attempting in such experiments as these to study the parallelism between two phenomena, of one of which we can obtain a relative measure only down to certain unknown limits of smallness, while of the other we can make no relative measure at all—we can, in fact, only assert with some probability that it is present or not.

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* *Cf.* Bernstein, 'Arch. f. d. ges. Physiol.,' 1902, vol. 92, p. 562.

On these grounds, in the ultimate resort, I believe every attempt to show directly a want of parallelism between the propagated disturbance and the electric response has been wrecked. Must we, then, abandon the attempt to enquire by direct experiment whether changes in the electromotive force of the electric response do run parallel to changes in the propagated disturbance? There is one possible method, and, as far as I can at present see into the matter, only one, by which it is possible to obtain a relative measure of the propagated disturbance, that is to measure it in terms of the only property which we can assign to it without unwarranted assumption, its ability to propagate itself. It is this method which Adrian and I used when we were trying to measure the effect upon a propagated disturbance of the time-interval separating it from a predecessor which had passed along the same path. We found that the shorter the interval the less was the distance which the second disturbance could travel without extinction through a tract of nerve in which it was undergoing a decrement. The essence of the method is to set the disturbance, whose intensity is to be measured, at a region of nerve where conduction is impaired, and to determine whether it is there capable of travelling as far as a full normal disturbance could travel. A comparison of the distance travelled by the disturbance under test with the distance travelled by the normal disturbance gives a measure of the relative intensity of the former. Another example of the method is its use in the work of Adrian on the recovery of a disturbance after it has emerged from a tract of nerve where it has been undergoing a decrement. His method was to lead it, after it had emerged clear from one such tract, into a second, and to see whether it was as successfully propagated through the second tract as was a fresh disturbance which had not faced the first tract at all.

This is a possible method of obtaining the measure which we need. Whether the future solution of our problem will rest on the laborious accumulation, by some such method, of evidence that the electric response and the propagated disturbance are indissolubly bound together, cannot be foretold. Up to the present the available evidence does not contradict the proposition that the electric response is a constant concomitant of the propagated disturbance. But for the purpose of any hypothesis as to the physico-chemical nature of the latter, the mere stringent proof of that proposition would not be enough. The important point for any such hypothesis is whether they are identical, that is whether the disturbance of electric potential at one point on a nerve is the actual and direct cause of the same phenomenon in a neighbouring part. Any hypothesis must be prepared to state whether the electric phenomena play the central part in propagation or are to be relegated to the position of a mere by-product.

On this point it is hardly likely that the direct experimental method can decide. A more hopeful line of enquiry springs from the study of that local excitatory process whose recognition was the first step in this analysis. If we can satisfy ourselves as to the physico-chemical nature of the local change we shall know the conditions by which the propagated disturbance is initiated, and we shall have some ground for hypothesis as to the change which would need to be produced by the propagated disturbance in one part of a nerve in order that a like change might spread to the neighbourhood. To this point I shall return.

There is one more line of experimental work upon the relation of the electric response to the propagated disturbance which must be taken into account before we pass to the remaining steps of the analysis. Though the general fact of the association of the electric response with the propagated disturbance is already established clearly enough, yet there is evidence pointing to a modification of the electric response by the mechanical conditions of contraction in a muscle. Amaya,[*] Bernstein and Tschermak,[†] and more recently Samojloff,[‡] have called attention to alterations of the electric response consequent on the tension encountered by a muscle during contraction. These facts may have no more significance than an alteration of the electric conductivity of a muscle during contraction. They may, however, touch our argument more closely in the sense that, as Straub[§] has recently suggested in the course of work on the heart, some small part of the electric response of muscle may have its origin in chemical processes related to contraction. This possibility needs to be kept in mind even though the general origin of the electric phenomena in nerve and in muscle can scarcely be supposed to differ.

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[*] Amaya, 'Arch. f. d. ges. Physiol.,' 1898, vol. 70, p. 101.

[†] Bernstein and Tschermak, 'Arch. f. d. ges. Physiol.,' 1902, vol. 89, p. 289.

[‡] Samojloff, 'Arch. f. (Anat. u.) Physiol. Suppl.,' 1908, p. 1.

[§] Straub, 'Zeitschr. f. Biol.,' 1910, vol. 53, p. 499; *cf.* also Samojloff, 'Arch. f. d. ges. Physiol.,' 1910, vol. 135, p. 446.

I must now turn away from this dark question of the part played by the electric phenomena in propagation, and must trace the remaining steps of the analysis. It must be confessed with regret that just on that point which seems most fundamental to any hypothesis of the propagated disturbance the analytical evidence is the most obscure.

C. *The Refractory State.*

The refractory state is the last of the phenomena found in nerve whose position in the chain of processes must be examined. I am using the term refractory state in perhaps too wide a sense, meaning to include all those phenomena of impaired functional activity which are associated with recovery from the excited state. These include not merely the actual impaired excitability which shows itself as the complete inefficacy of a stimulus of any strength for a short time, and then as the need for a stimulus stronger than the normal by an amount diminishing as recovery proceeds. There are also the phenomena which seem at first sight to affect the propagated disturbance more directly, namely the fact that the electric response to the second of two stimuli occurs with abnormally long delay, a phenomenon which Gotch* was the first to attribute to a slowing of the rate of propagation, and the fact that the second of two propagated disturbances is of abnormally small intensity if it follows a predecessor closely.† All these phenomena pass away gradually as the time increases since the previous activity of the tissue, and it seems not improbable that they are all expressions of a single process of recovery. The problem of the present analysis is the relation of this recovery to the propagated disturbance. The phenomena all follow the passage of an effective stimulus, and might therefore represent the recovery either from some change associated with the propagated disturbance or from some local change produced by the exciting current at the seat of excitation only.

The evidence on this point may best be taken for each of the phenomena separately. For the actual refractory period Tschagowetz‡ has given expression to the latter view by including that phenomenon in his hypothesis of the nature of the local excitatory process. Gotch§ noticed, however, that the refractory period appeared to be little affected if the second stimulus, instead of falling on the same point as the first, fell on a part of the nerve which had only been traversed by the propagated disturbance which the first stimulus had initiated. This fact Bramwell and I have confirmed,[*] and there seems to be real evidence that the refractory state is a consequence of the passage of the propagated disturbance, and not a local effect. The delay of propagation was ascribed by Gotch[†] to both factors, the propagated change and the local effect. His evidence on the latter point was that prolonged stimulation of a nerve brought the seat of excitation into a condition which delayed the propagated disturbance. On attacking experimentally the relative importance of the two factors, in so far as they enter into the effect of a single stimulus, I found that the delay was practically identical whether the second stimulus did or did not fall on the same point as the first.[‡] From this experiment it follows that the delay observed in such a case is a consequence of some change left behind in the tissue by the propagated disturbance as such. It would seem that the local effect which Gotch described does not enter normally into the delay, being only a consequence of such prolonged stimulation as produces some abnormal alteration of the tissue. The delayed propagation falls therefore into line with the refractory state as part of the process of recovery from some change associated with the propagated disturbance. The third phenomenon, the reduced size of the propagated disturbance elicitable during recovery, does not seem yet to have been assigned experimentally to its position, but the close parallelism of its progress in time[§] with that of the refractory period is strong evidence that both are aspects of one process of recovery.

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* Gotch, 'Journ. Physiol.,' 1910, vol. 40, p. 250.

† Boycott, 'Journ. Physiol.,' 1899, vol. 24, p. 144; Adrian and Keith Lucas, 'Journ. Physiol.,' 1912, vol. 44, p. 93.

‡ Tschagowetz, 'Arch. f. d. ges. Physiol.,' 1908, vol. 125, p. 401.

§ Gotch, 'Journ. Physiol.,' 1910, vol. 40, p. 267.

The sum of this evidence is that in all these phenomena of impaired activity we have expressions of a single process of recovery from some change which is associated with the propagated disturbance. From what change precisely the tissue is recovering we do not yet know. It seems very probable that in these phenomena we measure the actual return of the tissue to the equilibrium position after its disturbance by that change which is the basis of propagation.

The most definite attempt to relate the refractory state to the progress of other phenomena of activity has been that of Tait.[||] He starts from the observation which many experimenters have made, that there is a general association between the duration of the refractory period and that of the electric response, the two phenomena changing together as we pass from tissue to tissue, or from one condition to another. Beyond this he points out a characteristic effect of the drugs yohimbine and protoveratrine, namely the association of a great prolongation of the declining phase of the electric response with a great lengthening of that part of the refractory state which is marked, not by complete inexcitability, but by the need of stimuli of abnormal strength. On these facts he founds the hypothesis that the period of complete inexcitability corresponds to the rising phase of the electric response, that of only relative inexcitability to the declining phase. The facts on which this hypothesis rests are of obvious significance, but the interpretation put upon them cannot be justified without a thorough simultaneous measurement of the phenomena. It will be enough here to recall one pair of measurements which can be gathered from the general literature of the subject, in order to indicate that Tait's hypothesis will need more stringent proof before it can be accepted. Bazett* found that in the sartorius muscle of the frog a fall of temperature of 10° C. prolongs the refractory period by more than 200 per cent. ; over the same fall of temperature and in the same tissue I found† that the ascending phase of the electric response is prolonged by not more than 70 per cent. If these results are valid the two phenomena, though of like duration at one temperature, cannot remain so when the temperature is changed.

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[*] Bramwell and Keith Lucas, 'Journ. Physiol.,' 1911, vol. 42, p. 495.

[‡] Gotch, *loc. cit.*

[†] Keith Lucas, 'Journ. Physiol.,' 1910, vol. 41, p. 400.

[§] Adrian and Keith Lucas, 'Journ. Physiol.,' 1912, vol. 44, p. 93.

[||] Tait, 'Quart. Journ. Exp. Physiol.,' 1910, vol. 3, p. 221.

We have much still to learn about the relationships of the refractory state. But its recognition as a part of the process of propagation cannot fail to be a significant fact for any hypothesis of the propagated disturbance. It is already suggestive that the effect of temperature change on the process of recovery should be so widely different from that produced on the rate of propagation. Maxwell‡ first showed that the effect of a fall of 10° C. is an increase of the time of conduction in slug's nerve of the order of 80 per cent., and values of the same order have since been found for amphibian nerve and muscle.§ To set against this we have Bazett's observation of more than 200 per cent. increase in the refractory period. This value needs to be checked for other tissues, and particularly for nerve, but we can scarcely doubt that it will prove a significant fact when we reach the point of formulating a definite quantitative hypothesis of the propagated disturbance.

D. *The Contraction Process.*

Up to this point the processes into whose position we have enquired have all been such as occur in nerve as well as in muscle. In turning now to contraction and to the liberation of heat we have to deal with processes which are apparently absent from nerve and are therefore on the first showing not essential to propagation. We must not dismiss the matter quite so lightly. The possibility that these phenomena really constitute a part of the propagation process, reduced in nerve to minimal dimensions, is not to be overlooked.

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* Bazett, *loc. cit.*

† Keith Lucas, 'Journ. Physiol.,' 1909, vol. 39, p. 207.

‡ Maxwell, 'Journ. Biol. Chem.,' 1907, vol. 3, p. 359.

§ Keith Lucas, 'Journ. Physiol.,' 1908, vol. 37, p. 112 ; Woolley, *ibid.*, p. 122.

Bose,* arguing from the general probability that in all the excitable tissues the fundamental phenomena are identical, each particular aspect being merely accentuated in accordance with functional requirement, was led to try whether nerve was able to contract. By an optical method he found that electric currents from an induction coil did cause the nerve to shorten. That this shortening was the analogue of muscular contraction was not proved, and Waller† was led by his own experiments to regard it merely as a result of the heating effect of the "stimulating" current.

Quite apart from such evidence, there is a large body of experiment showing that the propagated disturbance may continue in a muscle which has been rendered unable to contract. Biedermann‡ was the first to bring evidence of this nature. He reported that a muscle steeped for part of its length in distilled water was no longer able to contract in that part, but was still able to transmit a propagated disturbance. The validity of these experiments was energetically denied by Kaiser,§ but seems to have been established by the later work of Overton‖ and Härtl.¶ The experiments of Härtl are particularly valuable, because they were so devised as to exclude by special precautions the possibility that the apparent passage of a propagated disturbance through the non-contractile part of the muscle rested in reality on a spread of the exciting current to the part which remained normal. Härtl found disproof of such a possibility in the long delay which his experiments showed between the passage of the exciting current in the altered part of the muscle and the appearance of contraction in the normal part. The disturbance was evidently propagated slowly through the water-logged muscle. The danger which besets the interpretation of experiments of this type is, of course, that there may have been in the water-logged part of the muscle a central core of fibres not yet completely robbed of their ability to contract, yet too feeble to bring about a visible shortening of the whole muscle. Such fibres might have served to propagate the disturbance from the seat of excitation to the normal region. However, Härtl's experiments were made on thin muscles which had been for half an hour in the non-isotonic solutions, and such a source of error is therefore improbable.

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* Bose, 'Comparative Electrophysiology,' 1907, p. 507.

† Waller, 'Proc. Physiol. Soc.,' March, 1908; 'Journ. Physiol.,' vol. 37.

‡ Biedermann, 'Stzber. d. k. k. Akad. Wien.,' 1888, vol. 97, III, p. 101.

§ Kaiser, 'Zeitschr. f. Biol.,' 1895, vol. 31, p. 244.

‖ Overton, 'Arch. f. d. ges. Physiol.,' 1902, vol. 92, p. 146.

¶ Härtl, 'Arch. f. (Anat. u.) Physiol.,' 1904, p. 80.

Quite recently new evidence along the same lines has been brought to light by the work of Noyons* on the electric response of the heart. Working on the hearts of frogs, tortoises, and fresh-water mussels he has found that various drugs will abolish the beat in so far as it consists of contraction, while leaving the rhythmic electric response still strong. If in these hearts the disturbance is propagated along the muscular tissue, then the presence of the electric response in parts of the heart not contracting and not excited from without seems to be clear evidence that the propagated disturbance has passed over the muscle without any accompanying contraction. Mines† has also just reported a case of this dissociation. The skeletal muscles of the ray after treatment with a dilute solution of ether give no mechanical response to strong interrupted electric currents, but still contract fully when bathed with suitable concentrations of acid, alkali, or potassium salts. In this case the order of things is reversed, the mechanism of excitation and propagation being apparently thrown out of gear while the mechanism of contraction is still present. On quite different lines he has argued for the dissociation from the effects of certain ions on the heart. A hydrogen ion concentration slightly above that normally encountered by the heart will cause stoppage in the relaxed state; a still higher concentration produces permanent contraction. The separation of these two stages of action is emphasised by the fact that trivalent cations can produce the former but not the latter. He suggests that the first stage means a stoppage of the processes concerned in excitation and propagation, while the latter indicates that in the meantime the mechanism of contraction has not been damaged.

All this evidence tends in the same direction, that of showing the contraction process to be not an essential part, but a consequence of the propagated disturbance, following that disturbance only in those tissues in which the necessary mechanism is present.

E. *The Liberation of Heat.*

The last of all the chain of processes which I am attempting to bring within this analysis is the liberation of heat. Happily, since we are now concerned only with relating this process to the propagated disturbance, the evidence is clear. Helmholtz* showed that in a nerve the passage of a succession of propagated disturbances did not raise the temperature by as much as 1/1000° C. Rolleston† lowered the possible change of temperature to 1/5000° C., and within the present year Hill‡ has shown that the heat liberated by the passage of a single propagated disturbance along a nerve cannot be sufficient to raise the temperature of the nerve by more than about a hundred millionth of a degree. This is the upper limit which the experimental method can safely state not to be exceeded. Actually the most sensitive instrument can detect no change at all. Such a finding means that the large heat-liberation which occurs in muscle must be a part of the contraction process and not of the propagated disturbance. The validity of this inference is strengthened by another fact which Hill has demonstrated by experiment, that in a given muscle under varying initial loads and with varying degrees of contraction the heat liberated bears a constant ratio to the tension developed.

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* Noyons, 'K. Akad. v. Wet. te. Amsterdam,' November, 1908, and April, 1910.

† Mines, 'Proc. Physiol. Soc.,' May 18, 1912.

F. *The Importance of the Local Excitatory Process to Hypotheses of the Propagated Disturbance.*

With this conclusion we are brought to the end of the first part of our enquiry, the analysis of the phenomena concerned with the propagated disturbance. We emerge from this analytical maze with some clear gain at least. As far as hypotheses of propagation are concerned we can rule out of consideration the process of contraction and the liberation of heat. The refractory state, on the other hand, we have learned to regard as the measure of a recovery process intimately concerned with the restoration of equilibrium after that disturbance which is the basis of propagation. This recovery process, with its characteristic progress in time,§ and its marked prolongation by fall of temperature, will therefore have to be reckoned with in any physico-chemical explanation of the process of propagation. But perhaps the most significant step of the whole analysis has been that which has failed to give positive results, the attempt to discover how the electric response is related to the change by which propagation is effected. It is the most significant because it brings home to us most clearly the incompleteness of that knowledge on which any hypothesis of the propagated disturbance must be based. But it is just here that we may look for help from another result of our analysis, the recognition of the local excitatory process. This process, according to the view which I have laid before you, is a change localised at the point of stimulation, produced as the direct consequence of the passage of the stimulating current, and providing when of adequate intensity the conditions within a tissue which are necessary for starting the propagated disturbance. If this account of the process is correct we cannot doubt the importance of its recognition. We are able to vary in many ways the character of the stimulus used to provoke this change. If we are using an electric current we can vary its direction, its strength, its duration, and the time-relations of its rise and fall. From the conditions which these various factors must fulfil in order to produce an adequate local excitatory change we obtain the data by which to test any hypothesis of the physico-chemical nature of that change. In such an hypothesis, supposing it to have been eventually established, we have a knowledge of the conditions within a tissue which are necessary for producing a propagated disturbance, and so we have fresh data for testing any hypothesis as to the nature of the latter. Perhaps by investigation along such lines we may be brought to an hypothesis of the propagated disturbance whose acceptance will carry with it a solution of that riddle of the electric response which seems to baffle direct experimental observation.

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* Helmholtz, 'Arch. f. Anat. u. Physiol.,' 1848, p. 158.

† Rolleston, 'Journ. Physiol.,' 1890, vol. 11, p. 208.

‡ Hill, 'Journ. Physiol.,' 1912, vol. 43, p. 433.

§ *Cf.* Adrian and Keith Lucas, 'Journ. Physiol.,' 1912, vol. 44, p. 114, fig. 18.

In sketching such a possible course I fear that I am outrunning too far the sober limits of accomplished work. Nevertheless, some progress has been made towards the first step, the physico-chemical identification of the local excitatory process. In the time that remains to me I shall attempt to show what this progress has been, and to lay before you both the hopeful signs and the very real difficulties which have to be met.

It is an old idea that the essential effect of an exciting current on a nerve or muscle is to produce a polarisation within the tissue. But the birth of this idea as a definite hypothesis open to verification by quantitative measurement begins with the work of Nernst.* Attracted by the fact that alternating currents of extremely high frequency can be passed through the human body without exciting the nerves or muscles, he was led to test the old suggestion that such currents pass only through the surface layer of an electrolytic conductor. He found this view untenable, and proposed that the essential condition which an effective exciting current must fulfil might be the production, at some membrane within the excitable tissue, of a definite concentration of certain ions unable to pass the membrane. This supposition would explain the failure of high frequency currents, because the rapid alternation of direction would carry the ions away from each membrane again before they had sufficient time to reach a considerable concentration. There were available at this time the experimental measurements made by many physiologists upon the relation between the minimal effective strength of an exciting current and its duration, rate of rise, and rate of alternation. Nernst* worked out the mathematical consequences of his supposition as far as these relations were concerned, and proceeded to compare the calculated values with those observed in actual experiment.

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* Nernst, 'Gott. Nach. Mathem. physik. Klasse.,' 1899, p. 104.

We shall miss the whole meaning of Nernst's work, and shall attribute to it errors which it did not contain, if we do not set clearly before us from the beginning the limitations which he consciously made in his mathematical treatment of the question. Speaking of his theory he says,† "It is limited to stimuli whose duration does not exceed a certain value; probably, however, divergence will presently be found also in cases where the action of the stimulus extends only over an excessively short period of time." In accordance with this Nernst found, as have others who have applied his formulæ to experimental observations, that the agreement between calculated and observed values of an exciting current is good only for currents whose duration falls within a limited range. For example, his theory leads to the prediction that single exciting currents of variable duration will just suffice to excite if the product of their strength and the square root of their duration is kept constant; in practice it is observed that for durations above or below a certain range the strength of the current must be considerably greater than the formula would indicate. Some physiologists have seen in this fact a reason for rejecting Nernst's work.‡ It must, however, be remembered that Nernst was conscious of the intervention of disturbing factors, particularly where currents of long duration were used, and did actually discuss the nature of those factors, though he did not include them in his mathematical treatment. I hold still to the opinion which I expressed in 1908,§ that the hypothesis is of heuristic value to say the least, and that our first concern should be to gain further knowledge of the disturbing factors.

Accordingly I shall now lay before you, as far as I am able to see them, the points in which Nernst's hypothesis is incomplete, and shall then consider such amendments or extensions of the hypothesis as have been proposed, and the lines along which future work is suggested.

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* Nernst and Barratt, 'Zeitschr. f. Electrochem.,' 1904, vol. 10, p. 664; Nernst, 'Arch. f. d. ges. Physiol.,' 1908, vol. 122, p. 275.

† Nernst, 'Arch. f. d. ges. Physiol.,' 1908, vol. 122, p. 310.

‡ Einthoven, 'Arch. f. d. ges. Physiol.,' 1900, vol. 82, p. 101; Wertheim-Salamonson, *ibid.*, 1905, vol. 106, p. 120; Hoorweg, *ibid.*, 1908, vol. 124, p. 511; Hermann, *ibid.*, 1909, vol. 127, p. 208.

§ Keith Lucas, 'Journ. Physiol.,' 1908, vol. 37, p. 477.

There are four main difficulties in the simple hypothesis that the concentration of ions has to reach a certain definite value at a membrane which the ions cannot pass. The first of these is that of which I have already spoken, that the mathematical consequences of the hypothesis in the simple form worked out by Nernst are at variance with the behaviour of currents of long duration. The second is that no account is given of the well-known physiological observation that a current rising to its full value up a gradient of less than a certain steepness will never excite, no matter what the value it ultimately reaches. The third is that the hypothesis in its simple form does not forecast one of the most fundamental facts of electric excitation, namely, the occurrence of the excitation at the cathode when the current is made, and at the anode when it is broken. The fourth is that we have to postulate membranes whose location in the tissue we do not know.

These points I shall deal with singly as far as that is possible, but in several cases they seem to dovetail into one another, being probably expressions of a common property of the tissues. The failure of Nernst's formula in the case of single currents of long duration expresses itself in the fact that, whereas if the product of the current strength and the square root of its duration is to remain constant the current strength should always diminish when the duration is increased, in actual practice it is found that, beyond a certain value of the duration, the current strength remains constant no matter how far its duration is increased. This feature of Nernst's formula, to which many physiologists have devoted criticism, is, as he himself stated with all clearness,* merely the consequence of the assumption, made in the mathematical reasoning, that the two membranes at which ions of opposite sign are concentrated are at a distance from one another which may be regarded as infinite. This assumption was a perfectly legitimate simplification when the exciting currents under consideration were only those of very short duration, because in such short times the effect of the proximity of the two membranes—namely, the steep gradient of concentration of ions in the solution between the membranes, and the consequent tendency to equalise the concentrations by diffusion—would not make itself felt. For longer durations of current, Nernst pointed out that, of course, this factor would come in, and would prevent the strength of the exciting current from falling indefinitely with great increase of its duration. Hill† has since worked out Nernst's hypothesis with a view to currents of longer duration than those contemplated by Nernst, and has been therefore obliged to take account of the distance between the membranes. His results show that a formula can be deduced in this way, which certainly does fit the observed facts of excitation by long currents. On this count, then, there is nothing to be said against Nernst's hypothesis. It was worked out by Nernst for stimuli of a limited range of duration, and simplifications were therefore made which applied only within those limits. That the formulæ so deduced are not found to apply outside those limits can hardly be used, as some physiologists have used it, as an argument against the validity of the hypothesis. Physiologists were naturally anxious that any hypothesis should take account of the features of excitation by long currents, because these are of particular interest in relation to the differences between different excitable tissues. For example, Lapicque* and I† had found that various excitable tissues could be differentiated by the fact that the duration at which the exciting current first reached a constant minimum value was longer for some tissues and shorter for others. The physical meaning of this difference was a point of obvious interest in the evolutionary history of the excitable tissues. But the absence of these facts from Nernst's formula was not to be set down to a failure of his hypothesis, whose consequences had simply not been worked out for the current durations with which we had dealt.

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* Nernst, *loc. cit.*, pp. 279, 280.

† Hill, 'Journ. Physiol.,' 1910, vol. 40, p. 190.

We may turn, therefore, to the second of the points which I have mentioned, the failure of slowly rising currents to excite. Nernst fully realised the point and suggested that the failure probably arose from a chemical change, produced by the ions, and proceeding at such a rate as not to be noticeable when the concentration was completed quickly.‡ He went so far as to set down in an equation the way in which the increase of the necessary concentration of ions might be related to the rate at which the concentration was produced and to the nature of the tissue used. Naturally this disturbing factor was not embodied in the treatment of currents of short duration, because both theory and experiment indicate that for such currents it is negligible.§ However, Hill‖ has since worked out the consequences of a supposition which is practically that originally offered by Nernst, and his conclusion is that though the general facts of observation are predicted, it is the experimental data which are not yet of a kind to allow of the comparison of observed and calculated values.

So far then it seems that the suggestions originally made by Nernst do offer a possible explanation of the observed facts of long and of slowly increasing currents. But we must not overlook the consequence, that the original simple hypothesis has become profoundly modified. Instead of the constant concentration of ions which sufficed to explain the facts of excitation by short currents, we now postulate a concentration which must be greater the more slowly it is produced. And we are further burdened with a chemical change produced by the ions, and constituting the necessary condition for the initiation of a propagated disturbance. Also the distance between the membranes now enters as a factor of importance, and this involves a fresh difficulty, to which I shall return when I come to speak of the location of the membranes. Several suggestions have been made in the attempt to escape from these complications. Hill* proposed a possible partial permeability of the membranes, which might serve to reduce the concentration of the ions by leakage. This condition could not be treated mathematically, and is also open to another objection, of which I am about to speak, that it offers no help towards the understanding of the occurrence of excitation at the anode when the exciting current is broken. Lapicque† looked for a solution in the modification of Nernst's fundamental hypothesis. He postulated as the conditions of excitation first a constant difference, and later a constant ratio, between the concentrations at two points unequally distant from the membranes. It seems doubtful whether this hypothesis does really account for the failure of slowly increasing currents. Hill found that it would predict the failure of such currents only if of such strength that they could not have excited in any case. Lapicque and Petetin are unable to confirm this conclusion in their more recent work. But in any case it does not appear that the hypothesis is a real advance on Nernst's, since it leaves the question of the anodic excitation still untouched. If we must admit a complication of the hypothesis in order to explain the failure of slowly increasing currents, the advantage will obviously be on the side of such a postulate as will explain also the anodic excitation. This, as we shall see, Nernst's suggestion does achieve.

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* Lapicque, 'C. R. Acad. Sc.,' 1903, vol. 136, p. 1147; 1905, vol. 140, p. 801; 'C. R. Soc. Biol.,' 1903, vol. 55, p. 445 and p. 753; 1905, vol. 57, p. 501.

† Keith Lucas, 'Journ. Physiol.,' 1907, vol. 35, p. 310; 1907, vol. 36, p. 113.

‡ Nernst, *loc. cit.*, pp. 280, 281.

§ *Cf.* Gildemeister, 'Arch. f. d. ges. Physiol.,' 1904, vol. 101, p. 203; Keith Lucas, Journ. Physiol.,' 1907, vol. 36, p. 273.

‖ Hill, *loc. cit.*, p. 203.

The anodic excitation on opening an exciting current is one of the most significant facts in the whole of our knowledge of excitation. It has received very little attention from critics of Nernst's hypothesis, probably because it is so old and well established as to have become less interesting than quantitative relations more recently investigated. Yet it is a fact which we clearly shall not explain if we postulate, as the condition which an exciting agent must fulfil, merely the production of a certain concentration of cations. When a current ceases to flow the concentration of cations does, of course, rise at the anode by diffusion until their normal concentration is regained, but there is no rise of the concentration of cations at the anode above its normal level. The one feature which is common to the cathode when the current is first made, and to the anode when it has just ceased to flow, is an increase of the concentration of cations* above the value which occurred at each point immediately before. It would seem, therefore, on this showing that the condition to be fulfilled by an exciting agent is either an increase in the concentration of cations above its recent level or some change which such an increase would produce. Nernst† pointed out that the conception which he had introduced to account for the failure of slowly increasing currents supplied just this need. Hill‡ dealt with the same point, perhaps even more explicitly, but it seems that the explanations which they offered are fundamentally the same. In both cases the diminished concentration of cations produced at the anode during passage of the current leads the reversible chemical combination between the ions and the postulated substance to take up a new equilibrium. When the current ceases to flow the compound is suddenly faced with a concentration of cations greater than that with which it is at the moment in equilibrium. That is, of course, also the condition with which it is faced at the cathode when the current begins to flow. On this supposition, therefore, the conditions at the anode on break of the current are brought into line with those which normally occur at the cathode on make of the current, and if excitation is to occur at the one it should be expected at the other also.

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* Hill, *loc. cit.*, p. 208.

† Lapicque, 'Journ. de Physiol.,' 1908, vol. 10, p. 601; 1909, vol. 11, p. 1009; Lapicque and Petetin, *ibid.*, 1910, vol. 12, p. 696.

As far as I am aware, this supposition is the only one which has been offered up to now as a common solution of these two fundamental facts of excitation, the failure of progressive currents and the excitation at the anode. The postulate that the concentrated ions are concerned in a reversible chemical change may appear, as I have suggested, an additional burden on the hypothesis, but if their concentration is to be the condition which an exciting current has to fulfil, we must suppose that they initiate some change dependent on their concentration, and it may be necessary that we should at this stage include the nature of that change as part of the whole hypothesis. To say that the supposition now before us is or is not satisfactory is at present impossible. Such a decision must rest with quantitative experimental work which has not yet been done; the mere qualitative explanation of the facts is unable to decide between this supposition and a host of others which could no doubt be made. I have already recalled how Hill pointed out that the existing experimental work on slowly increasing currents has a deficiency which makes it useless for quantitative comparison with values calculated from hypothesis. In the case of the anodic excitation also, long though the qualitative fact has been known, the quantitative relations necessary for verification have scarcely been touched. I must therefore leave this side of the problem with no more definite conclusion than this, that the next step must be taken in the laboratory and not in the study. From the mathematical treatment of the problem experimental workers have learned where the deficiencies of their experimental data lie. If the mathematical work bore no other fruit than this it would not have been wasted, because whatever hypothesis may ultimately be accepted its verification will demand the same facts.

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* Or, of course, decrease in the concentration of anions.

† *Loc. cit.*, p. 281.

‡ Hill, *loc. cit.*, p. 222.

There is one point which must not be overlooked in connection with the study of progressive currents and of the anodic excitation. You will have observed that it was only when these phenomena were being studied that we touched upon the problem of the effect which the ions might be supposed to produce, as opposed to the simple question of their concentration. This fact seems to me to add to the cogency of the reasoning which calls for renewed quantitative experiment upon just these particular phenomena.

I turn now to the last of those difficulties of the Nernst theory which we foresaw, the location of the membranes. It is an old conception, more than thirty years old at any rate,* that an exciting current produces a polarisation at a membrane forming a sheath to the excitable cell; and it seems that Nernst† had in view some membrane so situated. Indeed, it would appear that such an arrangement of the membrane which hinders the passage of the ions must be postulated if we are to explain the localisation of the excitations at make and break of the exciting current in the immediate neighbourhood of the electrodes which serve as cathode and anode respectively. If, for example, we were to suppose in a nerve or muscle the existence of a series of transverse membranes placed across the direction of passage of the exciting current, excitation would occur on one side of each of these membranes when the current began to flow, on the opposite side when it ceased, and the situation of the electrodes on the tissue would no longer coincide with the point of departure of the propagated disturbance. This raises an apparent difficulty in the way of part of Hill's treatment of Nernst's hypothesis. Hill introduced into the equations the distance between the membranes, as of course he was obliged to do when dealing with currents of long duration. From his equations the effect of an alteration of this distance can be foreseen. Now if, as I have suggested, the postulation of transverse membranes is proved untenable by the known facts of the localisation of the excitatory process, we must, at first sight, suppose that the effective distance between the membranes is really the distance between the electrodes placed on the surface membrane of the cell. But Lapicque has shown, and some unpublished experiments of my own completely confirm his results, that the constants of the curve relating current strength to current duration, which should be changed by an alteration of the distance between the membranes, are not changed when the distance between the exciting electrodes is varied by a large amount. This point needs to be cleared up, but further discussion of it at the present time would serve no useful purpose.

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* Hermann, 'Hdbch. d. Physiol.,' Leipzig, 1879, vol. 2, I, p. 193.

† Nernst, *loc. cit.*, p. 277.

Apparently, then, the hypothesis requires a sheath membrane impermeable to certain ions. What evidence is there to support or to defeat such a supposition? Work on the osmotic phenomena of cells is not wholly convincing on this point. For example, that of Overton* on the permeability of muscle fibres certainly gives evidence of the presence of membranes only slightly permeable to simple ions, but the location of such membranes is rather a matter of general probability than of demonstration. The bulk of the evidence which really bears upon our problem comes from work on the electric phenomena of the excitable tissues. Ostwald† seems to have been the first to suggest that the presence of membranes permeable only to certain ions might be the determining factor in producing differences in the concentration of ions within and without the excitable cell; such differences of potential might be the source of the electric phenomena observed. Cybulski‡ also pointed out that the electric phenomena of injured excitable cells would result, if there were normally a permanent difference of potential between the inside and the outside of the cell. More recently he has returned to the problem,§ and, though postulating also additional sources of electromotive force, still gives to the existence of the permanent polarisation at a surface membrane the chief part in the electromotive phenomena. He constructs, in fact, with an artificial semi-permeable membrane a model polarised cell, which yields just such electromotive phenomena as can be observed in an injured nerve or muscle. Bernstein* measured the temperature coefficient of the electromotive phenomena in nerves and muscles, and found evidence that concentration cells formed the source of the electric phenomena observed. He put forward as the most probable origin of the concentration cell the presence of a surface providing a sheath to the cell relatively impermeable to ions of one sign, and the consequent formation of an electric double layer. Brünings† also dealt with the localisation of the electromotive force of excitable cells, and reached by a process of exclusion the position that the surface of the cell is permanently polarised owing to the existence there of a membrane relatively impermeable to some ions. The facts on which he relied were these: that with rare exceptions concentration cells consisting of solutions of electrolytes cannot give closed circuits capable of being connected in series so as to sum their electromotive forces without metallic conductors, unless they arise from the presence of membranes of selective permeability to the ions present; that the electromotive force obtained without membranes would be less than that actually found; and that excitable cells can be polarised and also show osmotic phenomena.

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* Overton, 'Arch. f. d. ges. Physiol.,' 1902, vol. 92, p. 115.

† Ostwald, 'Zeitschr. f. physik. Chemie,' 1890, vol. 6, p. 71.

‡ Cybulski, 'Bullet. internat. de l'Acad. des Sciences de Cracovie,' 1898, p. 231.

§ Cybulski, 'Arch. internat. de Physiol.,' 1912, vol. 11, p. 418.

It is true that we do not find anywhere in this work an actual demonstration that such a membrane or surface as the hypothesis of Nernst would postulate is positively located as a sheath to the excitable cell. The evidence is rather that not only for the local excitation process, but also for the electric response, the postulation of a membrane so located seems at present to offer the simplest account of the facts observed. This fact in itself is significant, for if we are to find the key to the electric phenomena of the excitable tissues in the presence of polarised membranes or surfaces, it does seem probable, quite apart from Nernst's specific hypothesis, that the same membranes should be concerned both in the electric response which accompanies the propagated disturbance and in the local excitatory change by which the latter is started. At the same time, we must not forget the indirect nature of the evidence on which the existence of such membranes is founded. We need, perhaps, to look very far into the future before we shall see the present histology of the cell amplified by that new histology whose duty it will be to locate within the cell surfaces of physico-chemical importance.

Of those difficulties then which we foresaw in the first review of Nernst's hypothesis, the want of agreement between calculated and observed values where long currents are used cannot be said to have any weight, until it shall be found that formulæ based on mathematical reasoning intended to cover the case of such currents shall also fail. In the same category we must probably place the question of condenser discharges and alternating currents. Into these cases, though much work has been devoted to them, I have found it impossible to enter without prolonging this discussion beyond all legitimate bounds. The problem of slowly increasing currents and of the anodic excitation prove, however, to be on a different footing. They not only call for the postulation of a disturbing factor which need not enter into the other cases, but are at present so weak in quantitative experimental work as to render the verification of any hypothesis quite impossible. And lastly the location of the membranes is a difficulty which has scarcely yet been faced. If in this account I seem to have laid stress rather on the difficulties which stand in the way of the hypothesis than on the measure of success which it has already achieved, it is because I believe that by such treatment we may best gain from the hypothesis the undoubted help which it has to offer, even in its present stage of incompleteness. To disregard its difficulties would be to reject the guidance which it can give to future experimental work.

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* Bernstein, 'Arch. f. d. ges. Physiol.,' 1902, vol. 92, p. 521.

† Brünings, 'Arch. f. d. ges. Physiol.,' 1907, vol. 117, p. 409.

With this I reach the end of the story which it has been my endeavour to lay before you. I am very conscious that within the compass of this lecture I have been able to trace out but one of the many lines of work which are converging towards an ultimate solution of the central problem, the physico-chemical identification of the nervous impulse. In the investigation of the colloidal state, to take but one example, there is much done already and much more which needs to be done before even the formulation of a definite hypothesis can begin. And yet even in the limited range of facts which I have tried to recount I find a conviction that we can no longer repeat truthfully the words of Biedermann which I have quoted. I do not find that we are now as far as ever from an understanding of the intimate nature of the nervous impulse. If I have been able at all to make my meaning clear, you will have realised that we may now claim to have passed through the first phase of ignorance, in which we merely admitted that we did not know, and to have reached the second phase of ignorance, in which we are recognising what precisely are the points on which our want of knowledge is most profound. In treating of the analysis of the complex of phenomena involved in excitation and conduction I found it necessary to lay stress upon particular defects of our experimental knowledge. And again when the hypothesis of Nernst was before us it was with its special difficulties rather than its achievements that we were occupied. Its value for the purpose of immediate work proved in fact to lie largely in its detection of specific points of weakness in our experimental data. The very fact that we have been concerned so largely with the gaps in our knowledge is, I believe, a hopeful sign.

Perhaps it is another sign of the same progress that we no longer hope to capture the nervous impulse by storm, but have set persistent siege to its outworks. It is because the outworks show signs of yielding that we are a little nearer to understanding the nervous impulse, though we have as yet formulated no hypothesis of its intimate nature. In our analysis of the phenomena concerned with propagation we did, in spite of conspicuous difficulties, find that there was a clear definition of the range of phenomena which must be taken into account. Nor must we overlook the importance of that step which led us to recognise the local excitatory process. In the physico-chemical identification of that process there seems to lie before us the next stage of our progress. In Nernst's hypothesis of the nature of local excitation it is my belief that we have a means of attack which is quite invaluable. It is not a complete theory, ready, or even approximately ready, for acceptance, but it is an indispensable guide to the strengthening of our experimental data, and so to the ultimate elaboration of an hypothesis which shall be free from those difficulties which are at present so obvious. Our concern in the immediate future must be with quantitative experimental work. The ultimate hypothesis to which such work shall lead may perhaps be very near to that which Nernst has stated. On that point it would be idle to speak at present. However that may be, when a wholly satisfactory theory of the local excitatory process is in our hands we shall have a new weapon of attack upon the physico-chemical nature of the nervous impulse.

And it is not only in this preliminary work on the local excitatory process that the hope of new progress springs from a closer definition of our ignorance. In direct work upon the central problem of the propagated disturbance the same is true. I have tried to show you how, in our work upon the phenomena of propagation, we have been hampered because we could get no quantitative measure of that disturbance, and I have attempted to discuss the possibility of obtaining such a measure in the future. We shall have made some progress to-day if we have done no more than realise the importance of that gap in our knowledge. For whenever the time shall come for the formulation of definite hypotheses of the propagated disturbance, it can hardly be doubted that the work of verification will rest in large measure, as it does in the case of every scientific hypothesis, on the comparison of calculated inferences with the results of quantitative measurement.

The Development of a Parasite of Earth-worms.

By John Westray Cropper, M.B., M.Sc., Liverpool.

(Communicated by Sir R. Ross, K.C.B., F.R.S. Received June 20, 1912.)

(From the Laboratories of the John Howard McFadden Research Fund, Lister Institute.)

[Plate 14.]

While carrying out experiments on the artificial induction of cell-division, I had occasion to examine the seminal vesicles of the common earth-worm, when I noticed that certain epithelial cells contained within their cytoplasm structures similar to those known as "Kurloff's bodies" found in the blood-cells of guinea-pigs.

These Kurloff's bodies have recently been shown to be parasites, to which the name of *Lymphocytozoon cobayæ* has been given. In their early stages, they are parasites of the mononuclear cells (lymphocytes) of the blood of the guinea-pig. As described in the paper on the subject (E. H. Ross, 1912), they undergo development in definite stages in the lymphocyte, and ultimately become free-swimming spirochæte-like bodies in the blood-plasma. Having assisted in the investigation of the development of these so-called Kurloff's bodies, I was struck with the similarity between them and the new structures seen in the epithelial cells of the seminal vesicles of the earth-worm, and the matter was further investigated. I am now convinced that these structures in the earth-worm also represent phases in the life-cycle of a new parasite which is very similar in its developmental figures to *Lymphocytozoon cobayæ.*

The cells in the worm which contain these peculiar structures are the large cœlomic epithelial cells which form the walls of the vesiculæ seminales of *Lumbricus terrestris.* The body first observed was a large spherical sac containing a closely woven skein, staining deep blue with azur stain, lying embedded within the cytoplasm of the epithelial cell. The method of examination employed was that devised by H. C. Ross (1910) for the examination of blood-cells *in vitro,* and it is known as the "jelly method." A 2-per-cent. solution of agar in distilled water is boiled, sterilised, and filtered. To 5 c.c. of the filtrate is added 1 c.c. of a 1-per-cent. solution of azur II, and the total bulk of the mixture is made up with water to 10 c.c in a test-tube. When molten, a small quantity of the jelly is allowed to spread itself in a thin film on a microscope slide, where it will set when it cools. A drop of a suspension of the cells of the seminal vesicles in citrated normal saline solution is placed on a cover-glass, which is then inverted and allowed to fall flat on to the set jelly film. The spermatozoa, the nuclei of the epithelial cells and of the seminal cells all stain deeply. Within the epithelial cells the parasites also stain in sharp contrast with the surrounding cytoplasm, so that their morphology can be readily determined. The first stage of the parasite within the cell consists of a blue dot or granule situated in what looks like a vacuole which colours only faintly (Plate 14, fig. 1). In the next stage observed, the dot has apparently divided, for the vacuole or sac now contains several blue granules (fig. 2). The dots then elongate into rods (fig. 3), and each rod becomes lengthened and splits longitudinally (fig. 4), until the parasite appears like a bunch of painted sticks—a sheaf embedded within the cytoplasm of the epithelial cell, its host. This process of splitting continues and at length there is a series of spiral coils wound up within the sac, which is still enveloped by the protoplasm of the epithelial cell (figs. 6 and 7).

In some specimens observed by the jelly method, the parasite is separated from its host-cell, and is found stained and lying free as in fig. 5. The final stage is that of a free spirochæte (fig. 8). This is the fully developed parasite, having a long slender body, pointed at one end and blunt at the other. It is nearly always found coiled up, but occasionally it is almost straight. Its protoplasm is homogeneous, and contains at irregular intervals in its length small chromatin masses, varying in number from five to twelve, which are characteristic of spirochætes. These spirochætes have not only been seen free, but also swimming actively in the seminal fluid when the latter has been examined by dark ground illumination. They are readily differentiated from spermatozoa by their length, granulation, and peculiar movements, nor can there be any confusion between this parasite and the well-known *Monocystis lumbrici* or the spermatophores. So far as I can ascertain, its existence has not been recorded before. It is interesting to note, however, that intracellular phases of spirochætes have been described in other animals by A. Balfour, H. B. Fantham, E. Hindle, Sir William Leishman, and others. Its resemblance to the parasite of the guinea-pig is very remarkable; the earth-worm parasite is larger however, develops in the fixed epithelial cells of the vesiculæ seminales, and has not been seen in the blood of its host nor in the cœlomic fluid, but it passes through corresponding phases. The parasite is present in about one earth-worm out of five.

Summary.

A description of "bodies" found within some of the epithelial cells of the vesiculæ seminales of the earth-worm. They closely resemble "Kurloff's bodies"

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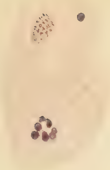

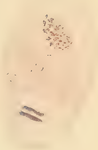

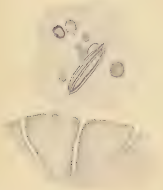

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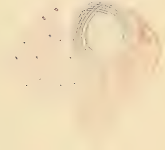

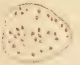

5

West, Newman chr.

OF A RASITE OF EARTHWORMS.

found within the lymphocytes of guinea-pigs, and by means of the jelly method of examination, the development of these bodies into free spirochætes is demonstrated in the same way that it has recently been shown that "Kurloff's bodies" also become spirochætes. I would suggest that these new parasites be called *Spirochœta lumbrici.*

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Notes on the Polymorphism of Trypanosoma gambiense *in the Blood and its Relation to the Exogenous Cycle in* Glossina palpalis.

By Miss Muriel Robertson.

(Communicated by the Tropical Diseases Committee of the Royal Society. Received July 5, 1912.)

I. *Introduction.*

The following paper deals with the well-known phenomena of the fluctuation in the numbers of trypanosomes present in the blood of an animal infected with *T. gambiense,* and with the equally familiar question of the polymorphism of the parasites.

The relation of these factors to the production of infected *G. palpalis* is also discussed, and evidence is brought forward to show that a certain type of trypanosome is responsible for the carrying on of the cycle in the transmitting host. The actual details of the structure of the trypanosomes and the sequence of developmental stages in the fly are not touched upon in this account. They will, I hope, form the subject of a subsequent paper.

II. *General Condition of a* T. gambiense *Infection.*

It is advisable to consider first the fluctuation in the numbers of the parasites. It is important to note here that a close study of any given infection brings out very clearly that the multiplication of the trypanosomes occurs in the circulating blood-stream. Search has also been made for any type of multiplication in the cells of the lung, liver, and spleen, so far entirely without success. Moreover, the invariable correlation between a rise in the number of trypanosomes and a rise in the percentage of dividing forms renders such a development very improbable.

The factors controlling the numbers of flagellates present in the blood at any given time fall naturally into two categories.

(1) Those conditioned by the vertebrate host.

(2) Those conditioned by the parasite.

Phagocytosis, alterations in temperature and the liberation in the serum of protective substances (possibly also any alteration in the viscosity of the blood) constitute the more important reactions on the part of the host likely to influence the condition of the trypanosomes, to act as inhibiting circumstances to the multiplication and to cause reduction in the number of the parasite.

The important factors in regard to the trypanosome itself are the self-conditioned processes within the body of the parasite and the production of toxic substances due to its metabolism.

By self-conditioned processes I mean such manifestations as the inherent capacity for division and nutrition and the general vigour and adaptability of the parasitic organism. The actual reduction in the numbers of the trypanosomes sets in as soon as the nugatory conditions, whatever their origin, get the upper hand. It is obvious that a continual state of tension exists between the capacity of the host to destroy the parasite and the capacity of the parasite to maintain itself. If these facts are borne in mind the nature and the details of the fluctuations are more easily comprehended, though it may not be clear exactly which set of factors is at work.

The two sets of factors here mentioned form, it is clear, merely two general groups of conditions; each group represents obviously a very complex aggregate of interacting circumstances concerning which we have at present practically no knowledge.

Thus, so far from knowing what inhibits the multiplication of an organism, we have still, in spite of the very valuable work of Hertwig, Loeb, and Klebs, very little knowledge as to what are the conditions that determine the normal division of any cell. This last must be touched upon later. It is not, therefore, to be supposed that the unravelling of the conditions obtaining in an infected monkey does more than afford a certain clearness and precision to our view of the picture presented.

The drop in the number of trypanosomes is often sudden, but the completeness of the clearance varies within wide limits, and the duration of the depressed period is quite inconstant.

The complex factors at work producing such a reduction may supervene at any period as regards the absolute number of trypanosomes present, but the first indication that these factors have begun to come into operation is most often a reduction in the percentage of dividing forms. The actual disappearance of the trypanosomes is, moreover, generally preceded by a shorter or longer period, during which the numbers are relatively stable and the divisions few. There are, however, occasional exceptions to this rule, and the numbers may suddenly be checked and reduced in the middle of a rise.

The mechanism by which the trypanosomes are destroyed has not been studied, but the work of other observers all points at present, as far as I am aware, to phagocytosis. One must, however, consider in passing (if only to dismiss it) the possibility of the trypanosomes entering into the cells of the host, and undergoing some form of development at this period. All the evidence is against this assumption on account of the important fact that the trypanosomes reappear relatively gradually, and their appearance is always accompanied by active multiplication in the blood stream. This fact is also of interest in showing that the disappearance is a genuine destruction and not a mere withdrawal of the parasite from the peripheral blood.

Lysis in the blood stream is also a possible explanation of the method of reduction in the numbers. There is a certain amount of evidence in favour of this to be drawn from a consideration of the endogenous cycle. It will be discussed in a later section.

The essentials of the fluctuation just sketched are repeated interminably with monotonous similarity, the only features liable to variation being the absolute numbers of trypanosomes involved and the time coefficient. That is to say, the drop may leave a fair number of parasites in the blood, and the rise may occur without further reduction, or the drop may be very complete; it may be sudden or more gradual. The depressed period may be long or short. Thus also the rise may be more or less rapid; the exalted period may be longer or shorter; the whole process may take place with low absolute numbers, that is to say, the exalted period may not involve a very high number of parasites—this last is often very characteristic of the later months of an infection. A stable or relatively level period may proceed to a rise instead of to a drop, owing probably to the removal of some temporary inhibition.

The extreme variability of these two elements, namely, the duration of time of the various periods and the absolute number of trypanosomes involved, produces a quite fictitious appearance of confusion. The essentials of the process are in reality extraordinarily constant.

As a matter of practical technique these points are more easily demonstrated in the earlier periods of the infection when the absolute numbers are relatively high. I have worked exclusively with monkeys, but no doubt the process is very similar in other animals infected with *T. gambiense.* Superficially, guinea-pigs and rats seem to behave as monkeys in regard to the fluctuations, but no detailed study has been made of them.

The coming and going of the trypanosomes described above may be considered to be the endogenous cycle in the blood of the vertebrate.

To consider now the polymorphism of the trypanosome and its relation (1) to the endogenous cycle, (2) to the production of infected flies, *i.e.* to the exogenous cycle in the fly.

III. *Method.*

The method employed in studying the trypanosome is as follows. Blood-films are taken daily from a *gambiense* monkey at the same hour (9 a.m.); are fixed by exposure to osmic vapour for half a minute; are immediately plunged into absolute alcohol and left for 10 to 20 minutes. Then they are dried in air and stained with Giemsa's fluid.

This method, while it gives the worst possible picture of the nuclear detail, has nevertheless excellent qualities in regard to the type of result at present required. To begin with, it fixes all the trypanosomes present in the drop of blood, which no true "wet method" does with absolute certainty; it flattens the creatures into one plane so that they may be more easily drawn, it is rapid, the errors are apparently very uniform, and it agrees with the method employed by other workers.

Whenever possible 100 trypanosomes were drawn with the camera at 2000 magnification. They were measured by means of a compass set at a distance corresponding to one micron, as in Bruce's method. The results were tabulated, and finally plotted out on squared paper and a curve drawn. In cases where the trypanosomes were very scarce 25 or 20, and in one instance 10, trypanosomes had to suffice; it is, however, obvious that in these cases the smaller numbers are quite as good a sample of the total forms present as 100 individuals on days when the parasites were more numerous.

The above method of measuring has been criticised by Dr. F. W. W. Stephens,* who recommends the projection of the trypanosomes on to a screen in a dark room and considers that the actual measurement should be done by the "tangent line method."

Drawing by means of the camera is obviously open to criticism, but the projection of the trypanosomes on to a screen is a counsel of perfection beyond the present achievement of workers in Central Africa. Dr. Stephens' objection to the compass method is that the last fraction has often to be estimated. This last criticism is not important in the present instance, since, for example, the point 19 on the squared paper simply implies that the length of the trypanosome falls between 18·50 μ and 19·50 μ. The minute fraction of a micron need not cause undue anxiety when it is remembered to what rigorous treatment a soft protoplasmic organism has been subjected in the obviously energetic process of fixing and staining.

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* 'Sleeping Sickness Bulletin 36,' 1912, No. 4.

Moreover, it is the relative and not the absolute lengths that are significant in the present connection, and, further, in no case do the important considerations turn on very slight differences of size. All possible care has been taken with the drawings, and it is hoped that the inevitable coefficient of human error will be compensated for and evened out by the relatively large numbers of individuals involved.

By comparing the daily curves the distribution of the different types of trypanosomes in relation to the endogenous cycle can be observed with considerable precision. Cages of newly hatched laboratory-bred flies were fed daily when possible upon the selected monkey, and the nature of the trypanosome infection at the time of feeding was further studied in relation to the number of infected flies produced. Each cage was fed only once on the infected monkey, and every possible care was taken to see that the flies had actually fed. To obviate individual idiosyncrasies in the blood of clean monkeys used to nourish the flies during the experiment, the monkeys were pooled, and every cage was fed on each of the group in turn during the first 15 days. The nature of the food does not seem to affect the production of infected flies after the first days.

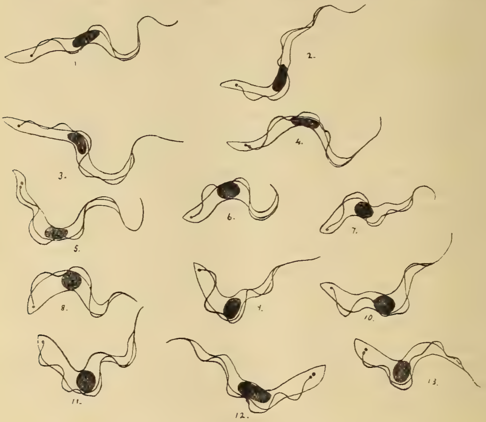
Owing to some other experiments being carried on at the same time in the laboratory, most of the cages were actually tested as regards infectivity, and produced typical infections in the test animals in every case in which *plus* flies were present.

IV. *Endogenous Cycle.*

Before considering the experiments in detail, it is best to give a brief account of the general results obtained.

T. gambiense varies in length from 10 microns to 34 microns. The minimum and maximum lengths are rarely reached, the bulk of the forms varying between 15 μ and 32 μ. There is really no sharp distinction into separated types, though the range of variation is relatively very wide. The trypanosomes may, nevertheless, be divided up readily enough into short, long, and intermediate individuals, but the intermediate forms insensibly join up the short and the long specimens. There is a continued transition from one type to another, but the duration of the different states varies considerably, and it is this variability of the time factor, coupled with the striking appearance of the individuals at either end of the scale, that has led to the belief that the species is really dimorphic. Such figures as 6–8 and 1–3, showing respectively short and long forms, have fostered this idea, and seem further to have suggested that the difference is an expression of sex. Neither of these hypotheses has, however, been sufficiently tested.

If one infection is carefully followed through a typical revolution of the endogenous cycle, the following conditions are found to obtain. It is convenient to choose the period immediately after a marked drop in the numbers, as the starting point. At such a time there are very few trypanosomes present in the peripheral blood, and they are short types.* As a matter of fact, the shortest trypanosomes of the cycle are to be found at this period.



Camera drawings. × 2000.

Figs. 1—3, long slender types. Figs. 4—5, typical early division stages. Figs. 6—8, short forms. Figs. 9—11, intermediate forms. Figs. 12—13, rare individuals of intermediate type in division.

* See Tables 18 and 9. The tables referred to are not reproduced here, but are reserved for publication in a forthcoming Report of the Sleeping Sickness Commission.

They have a short free flagellum, or may show practically no free flagellum, though it is never easy to be perfectly certain of the exact point where the body may be said to end absolutely and flagellum alone to exist. The breadth varies, but the forms are usually rather broad, measuring about 2 to 2·50 microns. In the course of the next few days there may be a level depressed period, with little or no increase in number, and there is correspondingly little change in type. As already said, the depressed period may be very short, or may last a number of days. The time factor, it must be emphasised, is open to the most capricious variation at all periods of the cycle. At the time when the first signs of a rise in numbers have occurred, it is found that with these there appears an immediate alteration in type, and long, slender, and intermediate forms are to be found, in addition to the short forms already present.

The sequence of events can be seen very clearly in cases where the depressed period shows a fair number of trypanosomes and where the rise is relatively slow; this is a quite frequent occurrence and the conditions are as follows:—

The first sign that a rise is about to take place is an increase in the size of a number of the trypanosomes; they become longer and there is a general increase in the length of the free flagellum. The width does not alter at first, and there is thus produced a state of affairs showing only short and intermediate forms, or if the increase in size is very general, intermediate forms may exist for the time being almost alone. This is an important moment in the cycle, as will be seen hereafter; some deeper physiological change in the trypanosomes accompanies the increase in size, or, rather, sets in at some period during the increase.

It is to be noted here that the rise in the numbers of intermediate forms (figs. 9–11) at this stage occurs at the expense numerically of the short forms, and one is, it appears, in every way justified in assuming that they are derived one from the other. There now occurs a marked drawing out of the body in a proportion of the parasites, and there are thus produced long, slender individuals such as that shown in figs. 1–3, and immediately upon this, the first burst of division occurs.

Naturally, if this process just sketched takes place very rapidly or very gradually, or with very low numbers, it is somewhat obscure, as the consecutive stages do not stand out with sufficient clearness.

As soon as the division actually takes place there is a reappearance or an increase, as the case may be, of the short forms. So that there are present long, short, and intermediate forms and dividing individuals. As the rise proceeds the relative numbers of these types vary, but there is always, as a glance at the tables will show, a relative decrease in the numbers of the long and intermediate individuals as soon as there is a lull in the number of the divisions. At the height the divisions cease or drop to a very low percentage, and correlated with this there is a reduction in the two types just mentioned. This disappearance of the long and intermediate forms is so marked that, just before the large drop in absolute numbers sets in, there is a state of affairs in which once more the shorter range of forms is almost the only one represented (see Table 16).

It is rather important to note that the main drop in numbers is usually preceded by a slight but quite definite numerical reduction which seems to set in after the divisions begin to slacken, so that it appears as though the inhibition of the divisions were already accompanied by a certain amount of actual destruction of the trypanosomes, though this is not yet serious in extent. This check in the divisions before the large drop in the numbers is evidence in favour of the serum exerting a harmful influence upon the parasites.

The inhibition of division can hardly be an effect of direct phagocytosis, and suggests that a certain amount of trypanolysis may occur in the circulating blood-stream. The vacuolated appearance of the trypanosomes to be noted in some cases just before or during the drop seems to lend further weight to this view.

Now one of the most striking points in the foregoing account is that the forms about to divide are the long slender ones. Figs. 4 and 5 give the typical picture of the earliest stages. The disappearance of this form when the divisions cease is also most striking in this connection, so that one is led to the conclusion that the long types are forms in preparation for division.

Briefly therefore my interpretation of the endogenous cycle is as follows:—The short forms (15–20 μ) constitute the normal adult blood type; this expression is merely used to indicate the form which has the largest duration in time in the cycle and which is the most stable. These increase in size and bulk, and form that sliding scale of individuals which may be termed the intermediate forms; these in turn lengthen out to the long, more slender types, which proceed to divide, giving rise once more to the short forms. The products of division are often unequal to a varying extent. One individual is usually much shorter, has a very short free flagellum or none at all, while the other partner may be of considerable length. Where this occurs there is a suggestion that the longer individual may proceed to divide again within a very short period, but the point is not very clear.

Early dividing individuals are found more nearly approximating to the intermediate type, but they are not numerous (figs. 12 and 13) and appear to be simply the representatives of the lower end of the range of size at which division occurs. In very numerous swarming periods or during very rapid rise they appear to be slightly more frequent and are probably due to a tendency to telescope the consecutive stages. It is to be observed that the kinetonucleus in these types not infrequently divides at right angles to the long axis of the trypanosome instead of parallel to it as is usual in the typical *gambiense* division. I am inclined to think that this may be an indication that the general tendency towards the drawing out in length has been suppressed in some way in these individuals. These divisions form only a very small part of the total numbers.

A sex interpretation of the above facts appears to me to be so improbable as hardly to merit discussion, and the arguments, as will be seen later, derived from the results of the glossina experiments are entirely contrary to such an hypothesis.

I am unable to suggest any reason why the trypanosomes should increase in length before division. We are at present without any clear knowledge of the internal stimuli and general factors producing division, although Hertwig's work upon the nature of the physiological tension existing between the nucleus and cytoplasm of certain organisms would possibly find an application in the present instance; but it is highly doubtful if that would afford any explanation of the alteration in the actual body form.

We know nothing as to what intensely complex factors really determine the body form of such an organism as a trypanosome. An intensive study of the physical and chemical condition of the blood at the different periods might reveal some external stimulus inducing the division of the parasites, and this might or might not afford the explanation of this particular change in shape. To maintain in the face of the facts brought to light by the study of the endogenous cycle that the long forms are males and the short forms females is obviously unreasonable. It would lead to such an absurd statement as that the males were responsible for the endogenous multiplication, the real fallacy being that we have at present no basis upon which to distribute the sex labels.

The point now arises as to whether there is any observable difference which might be attributed to sex among what I have termed the "adult types." There is a certain variation in length and breadth among these forms, but it is not marked, and there is no evidence so far as I have seen for attributing these trifling differences to sex. This leaves, of course, the question of actual conjugation quite unprejudiced for two very important reasons:—

(1) Flagellates both may and do conjugate without any external visible difference in the gametes.

(2) If differentiation of gametes does take place it may not occur until the forms are ingested by the transmitting host.

The next section, which deals with the infectivity to fly of the blood of these different phases of the endogenous cycle, affords further evidence in regard to the question of sex.

It may be pointed out in passing that a clear view of the endogenous cycle reveals a wide range of difference in the physiological state of the trypanosomes at different times, and it is clear that this is worthy of consideration from the therapeutical aspect of the trypanosome problem. The introduction of a drug would probably produce somewhat different effects, according to the moment of the cycle chosen for its application.

V. *The Relation of the Endogenous Cycle to the Production of the Infected Fly.*

The chief point brought forward by this aspect of the question may briefly be stated as follows :—

Is there any definite condition either of the individual parasite or of the infection as a whole requisite for the production of infected glossina ?

In a previous paper it has been recorded, from experiments treated in a different manner,* that negative periods in relation to fly occurred, although trypanosomes might be present in the blood at the time of feeding. Moreover, these experiments pointed towards the trypanosomes rather than the fly as being responsible for the negative result. The present work has confirmed this general observation.

Failure to infect fly must depend on some one or other of the following factors :—

(1) Failure of the flies to feed ; this may be called an adventitious negative, and can be obviated by keeping the flies for at least 24 hours after they have emerged, and by the exercise of patience and observation at the time of feeding.

(2) Absence of trypanosomes from the blood imbibed by the flies in the experiment.

(3) Absence of sufficient numbers of a given type (or types) of trypanosome capable of surviving in the fly.

(4) Presence of the requisite type, but in a physiological state unsuitable for survival in the fly.

(5) The capacity on the part of all the flies in the experiment of digesting all the trypanosomes imbibed.

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* 'Roy. Soc. Proc.,' B, vol. 85, p. 241, *et seq.*

It must here be mentioned that all the fly results are obtained in spite of a certain general tendency on the part of the flies to digest their parasites. All the experiments go to show that this negative factor may be taken as being very fairly constant. Any transmitting host whose digestion is rapid tends to show a relatively low percentage of carriers—thus mosquitoes, fleas, and tsetse flies, quite apart from the widely divergent nature of the parasites involved, all produce relatively few carriers.

Leeches and ticks, for instance, whose digestion is very slow in comparison, give, on the other hand, practically 90 to 100 per cent. of carriers.

It is advisable to take the same part of the cycle as that selected in the last section for the starting point, namely, immediately after a drop in numbers. Such a period which shows very few trypanosomes and all of the shorter type is an effective period and generally produces about the average number of *plus* flies. Now this is a result of considerable importance. There is at this time a population of trypanosomes which have just suffered a process of elimination, they have just passed through some set of conditions that has proved fatal to the vast majority. There are two things to consider: (1) the type of trypanosome which has survived, and (2) its condition. The type of trypanosome is very clearly the shorter individuals (see Tables 5, 17, 18), and they must, moreover, be those capable of resisting the particular adverse circumstances to which the majority have succumbed.

There is therefore at these periods a given type of resistant trypanosome which is, as shown by experiment, capable of infecting flies. It can therefore be concluded that not only are the short types the resistant individuals but also that this type by itself is capable of producing infection in flies.

The production of resistant strains by the use of drugs and sera has no doubt some relation to these probably temporary states of resistance occurring naturally in the untreated host. And it is not without significance to an understanding of the general biology of trypanosomes that these resistant individuals are also just those capable of carrying on the cycle in the transmitting host. This has a bearing too on the interesting discovery of the Commission of 1908, that trypanosomes persisting in antelope are rare in the blood but afford a relatively high percentage of infected flies. The low numbers and the absence of pathological symptoms on the part of the vertebrate imply that the conditions in the latter exercise a high degree of control over the parasite, and, correlated with this, the trypanosomes must in all probability be in a much more active state of resistance to their environment while sojourning in such a host. That this should also be the condition of affairs producing a large number of infected flies is of obvious importance and the connection with one another of the various instances just cited hardly needs to be further emphasised.

It has now been shown that the short forms are definitely capable of surviving in the transmitting host, but that in itself does not exclude the intermediate and long forms from a similar development. This apparently does not occur.

The days of the depressed period continue to be positive for fly until just before the rise, that is until the appearance of the intermediate forms in numbers sufficiently large to cause a serious diminution in the short forms. This period before the rise is one in which the percentage of *plus* flies produced sinks so low that none are found in the experiments—the number of flies ranges from about 45 to 100 in each experiment. In some cases this is probably an absolute negative, more often it will be only a relative negative, and if several hundred flies were fed probably an infected individual would be obtained.

The positive and negative periods in regard to the infectivity to fly shade insensibly into one another, thus there is a moment when the drawing out in length of the trypanosomes has begun which is still positive although the negative period supervenes in a short time. When divisions are actually taking place the blood is infective to fly just in proportion to the number of short forms present. A study of the tables and the analysis of the experiments given later on make this point clear. Such a multiplicative period may have a negative phase as soon as the proportion of intermediate and long forms preponderates unduly.

At the height the infectivity has a tendency to diminish, although the requisite form is present in large numbers. This is very marked in a swarming infection showing very numerous parasites, here the swarming period is often negative—it has been a common experience to feed cages even for two consecutive days on a monkey in this state without producing a single infected fly. The reason is pretty clearly that the trypanosomes are in an exhausted state physiologically, and force is added to this when it is noted that the actual period of a drop is negative.

This last fact is also an argument in favour of the surviving trypanosomes found in the depressed period having developed their resistance during the unfavourable time. That is to say these surviving trypanosomes found immediately after a drop are not, as it were, a separate type of trypanosomes of a resistant character, but are a certain number of the ordinary adult type which have been capable of adapting themselves to the unfavourable conditions at the time of their occurrence. Were this not the case, both the swarming period and the period of the drop would be as infective to fly as the depressed period.

The above observations upon the production of the resistant trypanosomes are only what one would expect upon the assumption that the Protozoa are capable of evincing reactions corresponding in some sort to the phenomena of immunity in the Metazoa.

The very first day on which trypanosomes are to be found in the blood of a newly infected monkey falls into exactly the same category as any other active period of multiplication, and is positive or negative to fly according to whether the adult type is present in sufficient numbers or not.

While in the actual experiments here described the negative periods do not seem to be of very long duration, nevertheless in feeding many cages on a population of monkeys at random these negative states have a profound influence on the results. Thus, in a previous group of experiments, out of 62 cages fed only 29 showed infected flies.

All the above results are, it is clear, entirely opposed to a sex interpretation of the dimorphism. If the long forms were males and essential to the development in the fly then the depressed periods would not be infective. The question of sex does not seem to me to require further discussion in relation to the endogenous cycle.

VI. *General Summary.*

1. *T. gambiense* is a polymorphic species with a continuous range of variation.

2. The shorter forms of 14—20 μ in length constitute the normal adult blood type.

3. The intermediate individuals measuring 20—24 μ in length are growth forms and lead to the long forms of 23—33 μ, which are those about to divide. The polymorphism of the trypanosome is thus due to growth and division phenomena and does not correspond to a sex differentiation.

4. The shorter forms are those destined to carry on the cycle in the transmitting host.

5. There are definite periods when the blood is not infective to fly although trypanosomes are present. Such periods are (*a*) just before an outburst of multiplication; (*b*) during the destruction of trypanosomes preceding a depressed period; (*c*) the summit of an exalted period involving very numerous trypanosomes—at such a time the parasites very frequently show signs of exhaustion; (*d*) certain periods of rapid multiplication when both the absolute and relative numbers of the shorter forms are low.

Further Contribution to the Study of the Inheritance of Hoariness in Stocks (Matthiola).

By Edith R. Saunders, Lecturer and late Fellow, Newnham College, Cambridge.

(Communicated by W. Bateson, F.R.S. Received August 1, 1912.)

In an earlier account, in which the results of intercrossing various strains of glabrous (= wallflower-leaved) Ten-week stocks are described, it has been shown that the results are capable of explanation on the supposition that a certain inter-relation exists between the factors causing hoariness and those concerned in the production of coloured sap.* We may briefly recall the main facts in regard to these two characters. It was found experimentally—

(1) That glabrous sap-coloured strains of various colours (*e.g.* dark purple, light purple, azure, red, flesh, copper) when intercrossed, produced offspring which were also *all glabrous sap-coloured;* later generations derived from these crossbreds were again invariably of this type.

(2) That these same glabrous sap-coloured strains, when crossed with the glabrous non-sap-coloured forms white or cream, gave *all sap-coloured hoary* in F_1; further, that self- or inter-bred F_1 plants gave an F_2 composed of the three classes, sap-coloured hoary, sap-coloured glabrous, and non-sap-coloured glabrous, in the proportion approximately of $9:3:4$. There were no non-sap-coloured hoary.

(3) That unions between the two glabrous non-sap-coloured forms white and cream also gave F_1 *all sap-coloured hoary;* in F_2 the proportion of forms was about 9 sap-coloured hoary to 7 non-sap-coloured glabrous. In this case sap-coloured glabrous were absent, as well as non-sap-coloured hoary.

The suggestions put forward at the time in explanation of the above facts were—

(*a*) That two factors (C and R) must be assumed to be present in all sap-coloured forms; in the absence of either or both the sap is colourless.

(*b*) That hoariness similarly is due to the presence of two factors (H and K), but that this second pair becomes ineffective under certain conditions (see (*c*)).

(*c*) That an inter-relation must exist between these two pairs of factors of such a nature that the hoary condition due to the presence of the H, K pair only becomes manifest in a zygote which also contains the C, R pair. Hence it follows that strains which lack C or R or both will be glabrous even though the factors H and K are both present.

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* 'Reports to the Evolution Committee of the Royal Society,' Report III, p. 38. For details of the experiments, see Reports I and II.

On these assumptions the statistical results which were obtained from various unions between glabrous sap-coloured and non-sap-coloured forms could be accounted for, and the composition of the several types employed represented in terms of these factors as follows:—

Glabrous cream........................	cRHK.
Glabrous white........................	CrHK.
Various glabrous sap-colours	CRHk.

It has since been pointed out by Doncaster that the supposition that *two* factors are required to produce hoariness in a zygote containing C, R introduces an unnecessary complexity; for if, as appears to be the case, the manifestation of hoariness in these strains is conditioned by the presence of the two sap-colour factors (C and R), then the existence of *one* additional factor is all that is required to account for the several results given above (see 1, 2, and 3). The justice of this criticism was acknowledged, and in the later accounts of these experiments Doncaster's simplification has been adopted, and the unnecessary factor (H) omitted from the formulæ of the several types. More recently, however, I have employed another sap-coloured form, and the results obtained with this new type make it clear that the earlier hypothesis that hoariness is determined by *two* factors, though not justified by the facts at the time it was put forward, is in fact correct, and consequently that the original scheme may be held to stand. This new type is a glabrous sap-colour known commercially as marine blue. Crossed with the original glabrous sap-colours, *e.g.* azure, flesh, and red, it gives *all hoary* in F_1, whereas these latter types, as stated above, give *all glabrous* when crossed together. On the other hand, when mated with glabrous whites from some new strains which had given, as before, *all hoary* with the original sap-colours, it gave an F_1 *all glabrous*. In short, marine blue gave results exactly the reverse of those previously obtained with other sap-colours. Reciprocal unions were carried out in both cases. We must, therefore, conclude that the marine blue strain lacked the factor K which is present in the other sap-coloured forms employed, but contained another factor not present in these other forms, a factor complementary to K in the same way that C is to R, the factor H referred to above, in fact, the postulation of which had not been warranted by the earlier results. The fact that two factors in addition to the colour factors C and R are required to produce hoariness being thus established, we can retain in their entirety the diagrams appearing on pp. 40 and 41 of Report III* representing the

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* See footnote, p. 540.

Table I.—Showing the Constitution of the 45 different kinds of Individuals which may occur in any *Non-sap-coloured* Glabrous Type.

[1 CrHK CrHK	[2 cRHK cRHK	[3 CrHk CrHk	[4 CrhK CrhK	[5 crHk crHk
[6 CrHK crHK	[7 cRHK crHK	[8 cRHk cRHk	[9 cRhK cRhK	[10 crhK crhK
[11 CrHK CrHk	[12 cRHK cRHk	[13 CrHk crHk	[14 CrhK crhK	[15 crHk crhk
[16 CrHK CrhK	[17 cRHK cRhK	[18 cRHk crHk	[19 cRhK crhK	[20 crhK crhk
[21 CrHK crHk	[22 cRHK crHk	[23 CrHk Crhk	[24 CrhK Crhk	[25 Crhk Crhk
[26 CrHK crhK	[27 cRHK crhK	[28 cRHk cRhk	[29 cRhK cRhk	[30 cRhk cRhk
[31 CrHK Crhk	[32 cRHK cRhk	[33 CrHk crhk	[34 CrhK crhk	[35 Crhk crhk
[36 CrHK crhk	[37 cRHK crhk	[38 cRHk crhk	[39 cRhK crhk	[40 cRhk crhk
[41 crHK crHK	[42 crHK crHk	[43 crHK crhK	[44 crHK crhk	[45 crhk crhk

composition of the F_2 generation in the particular cases there described, viz., where the forms employed are non-sap-colours containing both hoariness factors (H, K) with one or other of the colour-producing factors (C or R) and sap-colours containing one of the two hoary factors (assumed to be K*) together with the colour-producing pair C, R. But since any one of the four factors C, R, H, K can occur alone or with any combination of the others, and also with either white or cream plastids, it becomes possible by appropriate breedings to obtain a large number of individuals *identical in appearance* but *differing in constitution.* For, as shown in the accompanying table (Table I), we may have in the case of each of the glabrous non-sap-coloured forms (*i.e.* white and cream) as many as 45 differerent types of individuals, all alike to the eye and all breeding true: while a sap-coloured strain may include 20 different types (see Table II), also indistinguishable in appearance from one another.† Of these 65 types many have already been identified, and it is merely a matter of applying the cross-breeding test to a sufficient number of individuals in the F_2 generation to obtain the remainder, for by this method alone, at present, are we able to detect the presence of the non-effective factors and so ascertain the precise constitution of any particular individual.

Table II.—Showing the Constitution of the 20 different kinds of Individuals which may occur in any *Sap-coloured* Glabrous Type.

[1 CRHk CRHk	[2 CRhK CRhK	[3 CRHk CRhk	[4 CRhK CRhk	[5 CRHk CrHk
[6 CRhK CrhK	[7 CRHk cRHk	[8 CRhK cRhK	[9 CRHk cRhk	[10 CRhK cRhk
[11 CRHk crHk	[12 CRhK crhK	[13 CRhk CRhk	[14 CRhk crhk	[15 CRHk Crhk
[16 CRhK Crhk	[17 CRhk cRhk	[18 CRhk Crhk	[19 CRHk crhk	[20 CRhK crhk

The discovery of the fact that F_1 derived from two glabrous sap-colours is not invariably glabrous now makes it clear that no general statement can be

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[*] As we are unable to distinguish between H and K, the selection of K rather than H is, of course, purely arbitrary.

[†] Considered merely, *i.e.*, from the point of view of the two characters under consideration. If other characters are taken into account there would, of course, be many more.

made as to the result as regards surface character of matings between specified colours. Whether F_1 from two glabrous parents (dissimilar in colour or not) is all glabrous, or all hoary, or mixed, will depend upon the particular constitution of the parents in each case, and to a knowledge of this constitution, as we have seen, the colour of the flower is not in itself a sufficient guide. Nor can we predict the exact proportion of the different types in F_2 merely from a knowledge of the characters (colour and surface) in F_1, since we are unable by inspection to tell whether the crossbred is homozygous or heterozygous in respect of some of the effective factors, or whether again one member of a pair may be present, but ineffective owing to the absence of the complementary factor. We may, however, safely assert that where two glabrous individuals give a glabrous F_1 all the succeeding generations will be glabrous. On the other hand, where such an F_1 is hoary, F_2 will be mixed hoary and glabrous, the proportion of the two forms depending upon whether F_1 is heterozygous in 2, 3, or all 4 factors (C, R, H, K). If F_1 is heterozygous in only two of these factors we get a slight excess of hoary plants in F_2 (9 hoary : 7 glabrous). If F_1 is heterozygous in more than two factors we shall expect the glabrous individuals to be more numerous than the hoary. Other complications apart, the expectation will be 27 hoary : 37 glabrous when F_1 is heterozygous in three factors, and 81 hoary : 175 glabrous when it is heterozygous in all four.

We may now restate the main conclusions relating to surface character and the appearance of colour in the sap as follows :—

Summary of Conclusions.

1. Sap-colour in Stocks is due to the presence of two factors (C and R), in the absence of either or both of which the sap is colourless.

2. Hoariness also depends upon the presence of two factors (H and K) between which and the sap-colour pair a certain inter-relation may exist (see 3).

3. This inter-relationship between the two pairs of factors is such that when certain strains are inter-crossed the hoary effect due to the presence of the H, K pair is only manifested when the C, R pair is also present. Hence a *non*-sap-coloured individual may contain both H and K and yet be glabrous, but in a *sap*-coloured glabrous form H and K cannot both be present. One alone may occur, or both may be absent.

4. Similarly a *non*-sap-coloured glabrous form cannot contain *both* C *and* R. Either alone may be present or both may be absent.

5. Glabrous plants, whether alike in colour or not, when bred together, will yield an F_1 all hoary, or mixed hoary and glabrous, or all glabrous

according as the combined contribution of ovule and pollen grain includes *in every case* the four factors (C, R, H, K) necessary for the production of hoariness and sap-colour, or does so in the case of *only some* pairs of gametes, or in *none*.

6. No prediction can be made in regard to an F_1 thus bred until some cross-breeding test has been applied to the parents, since the result may be affected by factors whose presence in the parents is not revealed by their appearance, and which only become effective on crossing.

7. When F_1 from unions between glabrous plants is *all glabrous*, all later generations derived from F_1 will also be glabrous.

8. When F_1 from such matings is *all hoary*, F_2 will be mixed, the proportion of hoary and glabrous individuals depending upon whether the F_1 crossbred is heterozygous in 2, or 3, or all the 4 factors for hoariness and sap-colour.

9. The expectation in the case where F_1 is heterozygous—

In 2 of these factors will be an excess of hoary (9 hoary : 7 glabrous).
„ 3 „ „ „ glabrous (27 „ : 37 „).
„ 4 „ „ „ „ (81 „ : 175 „).

10. These same ratios may also appear in F_2 when a hoary and a glabrous plant are bred together, since here also the F_1 crossbred may be heterozygous in 2, 3, or 4 of the factors C, R, H, K. In this form of union, however, it is also possible for F_1 to be heterozygous in respect of only one of these factors, and in such a case the ratio in F_2 will be 3 hoary : 1 glabrous.

These experiments were carried out in one of the allotment gardens of the Cambridge Botanic Garden. The expense incurred during the progress of the work has been in part defrayed by a grant from the British Association for the Advancement of Science, and also from the Gordon Wigan Fund.

The Influence of Temperature on the Absorption of Water by Seeds of Hordeum vulgare *in Relation to the Temperature Coefficient of Chemical Change.*

By Adrian J. Brown, F.R.S., and F. P. Worley, M.A., M.Sc., New Zealand.

(Received August 9, 1912.)

Attention has previously been directed by one of us to the existence of a differential septum enclosing the seeds of Hordeum (barley).[*] When the seeds are immersed in aqueous solutions of most electrolytes, and of many non-electrolytes, this covering behaves as a very efficient differential septum, water alone entering the seeds under the attractive influence of the finely granulated contents.

The rate at which the water enters is considerably affected if substances are dissolved in it, being increased by some and diminished by others; it is also markedly dependent on the temperature of the water or solution in which the seeds are immersed. Variations of the rate at which water enters with alterations of the experimental conditions are presumably due mainly to changes in the water, and the seeds of Hordeum would thus appear to be a very suitable medium for the investigation of the nature of the changes produced in water by the presence of dissolved substances or by alterations of temperature.

In this communication, attention will be confined to the influence of temperature on the rate at which water is absorbed by the seeds of *Hordeum vulgare.*

Known weights of the seeds were immersed in water at definite temperatures constant to within 0·7° throughout the experiments. The mean temperatures at which observations were made are 3·8°, 21·1°, and 34·6°. After different intervals of time, the weight of the water absorbed by the seeds was ascertained by removing the seeds from the water, rapidly drying the surfaces, and then weighing. After weighing, the seeds were immediately returned to the water, and soaked during a further period.

The results of one series of observations are recorded in the following table, and shown graphically in the accompanying diagram (I).

It will be noticed that, during the earlier stages of the experiment, the rate at which the entry of water takes place increases very rapidly as the temperature rises; subsequently, when the saturation point is approached, and equilibrium is established between the seed systems and the water, the curves tend to run together towards the same asymptote.*

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[*] 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 82.

Table I.

Temp. 34·6°.		Temp. 21·1°.		Temp. 3·8°.	
Time.	Water absorbed.	Time.	Water absorbed.	Time.	Water absorbed.
hrs.	per cent.	hrs.	per cent.	hrs.	per cent.
5·00	16·80	5·25	9·32	5·58	4·42
24·00	35·64	24·33	24·80	24·75	11·82
48·25	43·44	48·50	35·98	48·83	18·52
71·50	45·36	72·00	43·42	72·25	23·42
96·00	45·74	96·25	46·90	96·50	27·42
				144·25	34·02

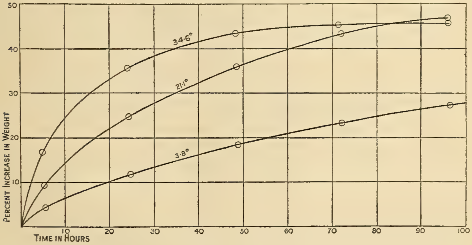

Diagram I.

In order to study more closely the effect of temperature on the velocity with which water is absorbed, in the first instance it is desirable to ascertain the relative rates at which water enters the seeds at the different temperatures. As the rate at any particular temperature decreases as the amount of water absorbed becomes greater, it is necessary, however, to ascertain the relative rates at different temperatures under comparable conditions.

As the conditions of the seeds throughout the experiments at the different temperatures are the same only when equal quantities of water have been absorbed, the rates of entry of water at the three temperatures were deduced at points where amounts of water corresponding to 5, 7·5, 10, 15, 20, and 25 per cent. increase in weight had been absorbed in each case. Higher percentages were not dealt with, on account of the possible influence of secondary changes in the latter parts of the experiments.

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* The slight decrease in the weight of the seeds at the highest temperature in the latter part of the experiment is due, in all probability, to secondary changes, perhaps in part to actual loss of weight through dissolution of solid matter, but more particularly to alterations in the attractive forces as water accumulates within the seeds.

The rates were deduced from the curves drawn carefully to express the result (Diagram I), by finding the values of the tangents at the required points by means of a stretched thread. The rates thus obtained, expressed in terms of the amount of water (percentages of initial weight) absorbed per hour, are given in the following table:—

Table II.

Water already absorbed.	Velocity.			$\frac{\text{Velocity at 21·1°}}{\text{Velocity at 3·8°}}$.	$\frac{\text{Velocity at 34·6°}}{\text{Velocity at 21·1°}}$.
	At 3·8°.	At 21·1°.	At 34·6°.		
per cent.					
5·0	0·506	1·72	4·50	3·40	2·62
7·5	0·416	1·51	3·83	3·63	2·54
10·0	0·356	1·27	3·07	3·57	2·42
15·0	0·282	0·897	2·30	3·18	2·56
20·0	0·216	0·679	1·68	3·14	2·47
25·0	0·164	0·570	1·16	3·48	2·04
			Mean............	3·40	2·44

If the sequence of ratios at the three different temperatures be compared, it will be seen that there is an approach to constancy in their relation; that this is the case is more clearly seen, however, on examination of the numbers in the fifth and sixth columns of the table, which express the proportional values obtained from a comparison of the three series of ratios.

Taking the mean values of the ratios, it appears that the velocities with which absorption takes place at the three temperatures 3·8°, 21·1°, and 34·6° are in the proportion of 1 : 3·40 : 3·40 × 2·44, *i.e.* 1 : 3·40 : 8·30.

The temperature coefficient is obviously high and of the order of that of a number of chemical actions occurring in solution. Moreover the velocity with which water is absorbed by the seeds is almost exactly an exponential function of the temperature. If this be so, the logarithms of the velocities plotted against the temperature should lie on a straight line. The results obtained on plotting the logarithms of the velocities given in Table II, including those of mean ratio of the velocities, are given in the following diagram.

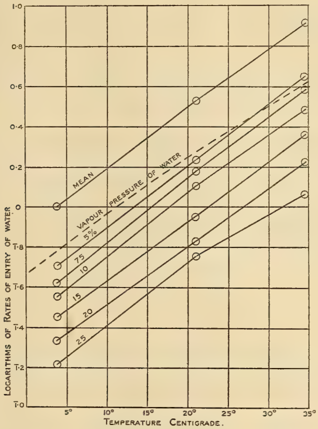

Diagram II.

The course of the lines in the diagram, in respect both of straightness and agreement of inclination, furnishes evidence of a most conclusive character that the rate at which water is absorbed by the barley seeds is an exponential function of the temperature.

Such a conclusion is of special interest, not only on account of the rarity of an exponential increase of a physical property with rise of temperature,* but more especially as the vapour pressure of water is also approximately an exponential function of the temperature.

In Diagram II the logarithms of the vapour pressure of water over the range of temperature covered by the absorption experiments are plotted against the temperature as a dotted line. The highly important fact is thus brought out that the slope of the curve is almost exactly the same as that of the curves representing the velocity at which water is absorbed by the seeds of Hordeum.

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* "An exponential increase of any physical property with rise of temperature is very rare. The increase of the vapour pressure of a liquid with rise of temperature is an exception and, in consequence, Arrhenius concludes that the increase of the velocity of a chemical reaction with temperature cannot be explained by any change in the physical property of the solution with temperature." Mellor's 'Chemical Statics and Dynamics,' 1904, p. 394.

Expressed in other terms, the relation between the temperature and either the rate of entry of water into the seeds or the vapour pressure of water may be approximately expressed by the equation

$$v = ae^{k\theta},$$

in which v represents either the velocity of absorption of water or vapour pressure, and θ the temperature, a being a constant.

The value of k in the case of the absorption of water by Hordeum seeds, derived from the mean ratios of the velocities, is 0·069; in the case of the vapour pressure of water, it varies slightly, having the following mean values between the temperatures cited :—

0·071	between	0°	and	5°
0·069		5		10
0·066		10		15
0·063		15		20
0·061		20		25

So close an agreement in the general character of an exceptional property common to liquid water and its vapour is an indication that the phenomena may have a common origin. In the case of an exponential increase of the vapour pressure of water with rise of temperature, presumably we are dealing mainly with the influence of temperature on the proportion of molecules of "hydrone" $(OH_2)_2$, and, in the light of our experiments, when measures are obtained of the velocity with which the passage of water takes place at varying temperatures through a differential septum under the attractive influence of minute particles, we may be obtaining measures of the activity of the simple molecules existing in the water. It would appear that only these simple molecules are directly assimilated by the seeds or transmitted by the differential septum.

The above results appear to throw light on the cause of the high temperature coefficients of chemical interactions occurring in solution. In a number of instances it has been shown that the rate of chemical change is approximately doubled by an increase of 10°. It is noteworthy that the value of k calculated above for the rate of absorption of water by the seeds of Hordeum corresponds almost exactly to a doubling of the rate for a rise of 10°, for which the exact value of k would be 0·0693. It appears highly probable that the velocity of chemical interactions occurring in aqueous solution is mainly dependent on the activity of water as expressed by the proportion of "hydrone" molecules. The importance of the part played by water and especially by the active hydrone molecules in chemical interactions has been frequently emphasised by H. E. Armstrong in various recent communications to the Society, and the above views are in complete harmony with the explanations recently advanced by Armstrong and Worley in discussing the nature of hydrolytic change.

Experiments with Aqueous Solutions of Ethylic Acetate.

Ethylic acetate belongs to the class of solutes (Hormones) which are capable of penetrating the seed covering, and it is further remarkable on account of its power of accelerating the velocity with which water enters the seed.*

In view of the results previously described in this paper it appeared desirable to study the velocity with which water is absorbed at different temperatures in the presence of the acetate.

Table III.

Temp. 34·6°.		Temp. 21·1°.		Temp. 3·8°.	
Time.	Water absorbed.	Time.	Water absorbed.	Time.	Water absorbed.
hrs.	per cent.	hrs.	per cent.	hrs.	per cent.
4·50	19·38	4·75	11·82	5·08	5·28
23·50	38·48	23·83	29·22	24·25	15·12
47·75	45·26	48·00	41·12	48·33	23·58
71·00	45·98	71·50	44·42	71·75	29·32
95·50	44·06	95·75	45·58	96·00	34·16
				143·75	40·84

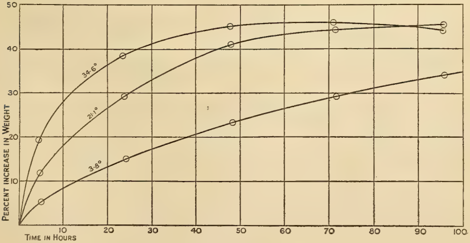

Diagram III.

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* *Loc. cit.*, p. 92.

A series of experiments was made in which seeds of *Hordeum vulgare* were steeped in "weight normal" solutions of ethylic acetate at different temperatures as in the experiments previously described. Curves representing the rate of absorption in these experiments are plotted in the diagram (III) from the values given in the preceding table.

It will be noticed that the curves resemble very closely in form the water curves (Diagram I), but their general course tends to run at a higher level, indicating an acceleration in the velocity of absorption in the presence of the acetate.

The values expressing the velocity of entry of water into the seeds were determined, as in the earlier experiments with water alone, from the curves at points representing 7·5, 10, 15, 20, and 25 per cent. of water absorbed. The values so obtained are given in the following table :—

Table IV.

Water already absorbed.	Velocity.			$\frac{\text{Velocity at } 21\cdot1^\circ}{\text{Velocity at } 3\cdot8^\circ}$	$\frac{\text{Velocity at } 34\cdot6^\circ}{\text{Velocity at } 21\cdot1^\circ}$
	At 3·8°.	At 21·1°.	At 34·6°.		
per cent.					
7·5	0·575	2·12	5·25	3·69	2·48
10·0	0·505	1·72	4·27	3·41	2·48
15·0	0·406	1·23	3·26	3·03	2·71
20·0	0·337	0·99	2·18	2·94	2·77
25·0	0·274	0·76	1·55	2·77	2·04
			Mean	3·28	2·50

On plotting the logarithms of these values the results shown in Diagram IV were obtained.

It will be noticed how closely the lines approximate to straightness, indicating that the velocity of absorption of water from a solution of ethylic acetate is an exponential function of the temperature as in the case of the experiments with water alone. Furthermore, as in the previous case, the slope of the lines is practically the same as that for the logarithms of the vapour pressure of water plotted as a dotted line in the diagram.*

The only noticeable point in which the results obtained with "weight

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* The form of the curves in Diagrams I and III is very approximately hyperbolic. Making use of this fact, it is possible, by plotting against the time the logarithms of the amount of water finally absorbed minus the amount absorbed at any given time, to arrive at the relative initial rates of absorption. Proceeding in this way, results similar to those described above are obtained.

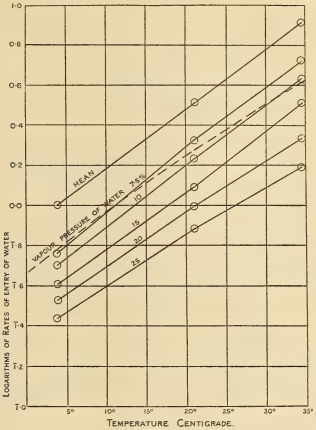

Diagram IV.

normal" solutions of ethylic acetate differ from those obtained with water is in the actual velocity with which absorption takes place under corresponding conditions. This is higher in the presence of ethylic acetate than in the presence of water alone. The temperature coefficient is apparently unaffected. Viewed in the light of previous remarks, this observation suggests that acceleration of the velocity of absorption in the presence of ethylic acetate may be due, in a large measure, at least, to the simplifying action of the solute on the molecular structure of water. If this be the case, it seems probable that the partial pressure of the vapour of water should be increased in the presence of small proportions of ethylic acetate or, in any case, that the lowering of the vapour pressure through osmotic effects should be in part counteracted by an increase due to the simplifying action of ethylic acetate and such substances as butylic and amylic alcohols. Investigation of this question is proposed by one of us.

Some Observations on Trypanosoma pecorum (*Bruce*) *and* T. uniforme (*Bruce*).

By H. L. Duke.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received August 16, 1912.)

The fact that antelope play a considerable part in nature as a reservoir for *T. gambiense* has now been thoroughly established. Both laboratory and field results agree in confirming the suspicion which for some time past has attached to these animals. In the case of *T. gambiense,* however, there is nothing very remarkable in the observation that the presence of the trypanosome in their blood apparently exerts no harmful effect on the antelope. *T. gambiense* cannot be considered as a fatal trypanosome for ruminants generally. In the case of *T. pecorum*, however, matters are different. This trypanosome, according to Bruce and his collaborators, causes a rapidly fatal disease in cattle and domestic animals generally. Though apparently widely distributed throughout Uganda, nothing definite is at present known concerning its true carrier in nature. The presence of the disease in districts where tsetse are unknown shows that some other agent is capable of conveying this trypanosome. The behaviour of laboratory-bred *Glossina palpalis* as carriers is so uncertain that this fly would appear at most to be a facultative host, though doubtless under favourable circumstances it may play an important part in nature. Again, nothing is known concerning the existence of a natural reservoir for this trypanosome in Uganda, though Kleine has recently obtained species closely allied to, if not identical with, *T. nanum* and *T. congolense* from antelope in the neighbourhood of Tanganyika. The following experiments were undertaken with a view to discovering whether *T. pecorum* is pathogenic to antelope, and at the same time to test the power of these animals to act as a reservoir for the trypanosome:—

A young bushbuck, born at the laboratory in January, 1911, was inoculated with blood of a monkey suffering from *T. pecorum.* After an incubation period of 15 days trypanosomes appeared in the blood of the bushbuck on May 31, 1911, being present for some days and then disappearing. Clean laboratory-bred *G. palpalis* were then fed upon the bushbuck and afterwards on clean monkeys. These experiments are given in Table I. As will be seen, in only one instance was a positive transmission obtained, and this was unfortunately not entirely satisfactory, as will be explained below.

Table I.

Expt. No.	Period for which flies fed on Bushbuck 55.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
112	June 6—13, 1911 ...	42	32	39	1	62	—	
129	June 9—13, ,, ...	37	34	37	1	63	—	
135	June 12—14, ,, ...	34	33	34	0	80	—	
247	Aug. 5—12, ,, ...	93	85	90	8	141	—	
253	Aug. 7—12, ,, ...	105	86	91	1	66	+	Fed on Monkey 359, which developed *T. pecorum*. *Cf. infra.*
541	Dec. 1—8, ,, ...	109	56	109	0	61	—	
542	Dec. 4—8, ,, ...	94	39	94	0	58	—	

The following cycle experiments were carried out in which animals other than Bushbuck 55 supplied the infecting feeds :—

Table II.

Expt. No.	Period for which flies fed on infected animal.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.		
96	1911, May 31—June 5, on Monkey 152	46	44	46	1	49	—
212	1911, July 19—21, on Calf 33 ...	83	80	82	2	104	—
368	1911, Sept. 6—9, on Monkey 321	136	124	133	1	108	—
716	1912, April 18—27, on Calf 688	46	42	45	2	82	—
717	1912, April 18—27, on Calf 688	59	53	59	2	82	—

It is unfortunate that in the case of the only (apparently) successful transmission experiment (253) a possible alternative source for the trypanosome in the monkey was introduced. Owing to a temporary shortage of monkeys monkey Experiment 359, whose blood had been negative for 10 days after the last feeding of Experiment 253 upon it, was taken to the Lake. Box 253 had been dissected, and as no fly was found with flagellates in the proboscis, it was thought that the experiment would necessarily be negative. It happened that three bushbuck, A, B and C, were shot, and the citrated blood of two of them, A and B, was inoculated into the monkey, 3 c.c. from each animal. At the same time, 6 c.c., 5 c.c. and 3 c.c., citrated blood of Bushbuck A, B and C, respectively were inoculated into Goat 484. This goat remained healthy, and examination of two blood films from each of A, B and C proved negative. Trypanosomes identical morphologically with *T. pecorum* appeared in the blood of Monkey 359 on November 13, 1911, 23 days after the inoculation of the bushbuck blood and 33 days after the last feed of Box 253. From November 1 to 10 inclusive the monkey was not examined. The trypanosomes might thus have been derived either from the bushbuck or from the positive fly of Experiment 253; in each case with a long incubation period.

The negative evidence of Goat 484 and of the blood films, together with the fact that such a trypanosome has never before been recovered from wild flies in the Mpumu district, makes it almost certain that the trypanosomes in Monkey 359 came from Fly 9 of Table III. The curious inability of laboratory-bred flies to infect monkeys with *T. pecorum*, even though showing a well marked proboscis infection, has been mentioned by Fraser and myself, and is also evident in Table III.

It is thus possible that the unusual swarming condition of the sucking stomach to be described in Fly 9 of this table may have a definite developmental significance in *T. pecorum*.

It may be noted that the trypanosomes in the monkey were characterised by a large vacuole posterior to the trophonucleus. This was very striking in the fresh state, even with low magnifications. This condition was lost in a subinoculated white rat.

A further observation which to some extent provides a parallel for the long incubation period, supposing Fly 9 to have been responsible for the infection, is afforded by Experiment 644. In the case of this monkey, inoculated from a goat suffering from *T. pecorum*, trypanosomes first appeared in the monkey's blood 26 days after inoculation. The blood in this case was examined daily. It is to be noted also that *T. pecorum* may often not be seen for days in monkeys' blood, and when present is frequently in very scanty numbers.

The curious backward condition of Fly 12 is interesting. Similar instances occurred in flies infected with *T. nanum*,[*] but then there was a possibility of a secondary "pick up" of trypanosomes from the calf employed. In the present instance, as the monkey of Experiment 247 never became infected, this explanation is not available.

It is to be regretted that in only three cases were the salivary glands obtained in the above positive flies. In all these the glands were negative. In this respect also *T. pecorum* shows resemblances to *T. nanum*.

A remarkable feature of the above table is the fact that no proboscis infections were obtained before the 76th day of an experiment. This delay

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[*] "Transmission of *T. nanum*," 'Roy. Soc. Proc.,' 1912, B, vol. 85.

Table III.

Date.	No. of fly.	Age of fly when dissected.	Sex.	Expt. No.	Region of gut.						Injection.
					Hind.	Thoracic gut up to proventriculus.	Proventriculus.	Sucking stomach and duct.	Salivary glands.	Proboscis.	
1911.											
July 15 …	1	9	♂	112	+ +	–	–	0	0	–	
Aug. 16 …	2	11	♀	247	+ +	–	–	0	0	–	
„ 29 …	3	24	♀	247	+ + +	+ +	–	0	0	–	
Sept. 19 …	4	45	♂	247	+ +	+ +	+	0	0	–	
„ 19 …	5	45	♀	247	+ + +	0	0	0	0	–	
„ 4 …	6	45	♂	212	+ + +	+ + +	+ + +	0	–	–	
July 19 …	7	49	♀	96	+ + +	+ + +	–	0	0	–	
Aug. 11 …	8	63	♀	129	+ +	+ +	+ + +	0	0	–	Contents of gut and proventriculus injected into 2 white rats = negative.
Oct. 10 …	9	64	♀	253	+ + +	+ + +	+ + +	+ + +	–	–	
„ 14 …	10	70	♂	247	+ + +	+ + +	+ + +	0	0	–	
Nov. 21 …	11	76	♀	368	+	+ +	+	0	0	+ + + attached long crithidia	
„ 3 …	12	90	♀	247	+ + +	–	–	0	0	–	
„ 2 …	13	104	♀	212	+ + +	+ + +	+	0	–	+ + 2 clusters fixed	
Dec. 5 …	14	122	♂	247	+ + +	+ + +	–	–	0	+ + long unattached	
„ 26 …	15	141	♀	247	+ + +	+ + +	+ +	–	0	+ + + long unattached	

In the above table the negative sign implies absence of flagellates; the sign 0 means that no observation was recorded.

on the part of the flagellates in reaching the proboscis became evident during the dissection of the earlier experiments and led to the carrying on of the later boxes for a considerably longer period. In spite of this, however, although four flies were obtained showing a good infection of the proboscis, none of these proved capable of transmitting the trypanosome to a healthy monkey. The only successful transmission, as has been pointed out above, was apparently caused by Fly 9, which showed a swarming infection of the sucking stomach and its duct. This inability on the part of the proboscis-infected flies to infect a monkey is remarkable. On several occasions the flies were starved for short periods and then again fed upon the monkey; also with negative results.

In the case of Fly 9, supposing it to be the infecting agent, two possibilities must be considered. Either the flagellates of the sucking stomach were responsible for the infection, or the proboscis may have shown a temporary infection which at the time of dissection had disappeared.

From the above experiments it will be seen that Bushbuck 55 was still capable of infecting laboratory-bred *G. palpalis* some three months after its original infection with *T. pecorum*.

The following experiments were carried out 10 months after the original infection of the bushbuck.

Date.	Expt. No.	Quantity of blood.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.			
21.3.12………	670	2½		—	Monkey.
23.3.12………	678	6		—	,,
3.4.12 ………	688	3½		+	Calf.

In considering the positive result of Experiment 688 the following facts may be quoted as excluding any source of error:—

(1) Since my arrival in Mpumu in September, 1910, there has been no case of spontaneous infection with this trypanosome among the laboratory cattle.

(2) The cattle in the neighbourhood of the hill are apparently free from the disease.

(3) A calf of about the same age as Experiment 688 (both were born at the laboratory) has been under daily blood examination from the beginning of April until the date of writing, and has never developed trypanosomes. These two animals have never been allowed to leave the hill top, and have always been stalled together.

It will thus be seen that no local epidemic of *T. pecorum* disease, such as affected the transmission work of the 1908—1910 Commission, interfered with the above experiments.

The interesting result obtained in Experiment 688 proves that *T. pecorum* can exist in antelope for at least 323 days. During this time it apparently exerts no harmful effect upon the host. The bushbuck, Experiment 55, is to all appearances in excellent health, and has been growing rapidly throughout the period covered by these experiments.

An interesting point also is the rapid course of the disease in Calf 688, which died in 51 days. Previous to the introduction of the trypanosome into the bushbuck there were some signs that the organism was losing its influence, possibly owing to continued maintenance in laboratory animals. Thus Calf 33, which was inoculated with the old laboratory strain of *T. pecorum* from a monkey, first showed trypanosomes on May 13, 1910. The animal is still alive and in good condition, and was showing *T. pecorum* in its blood on August 29, 1910; since then the blood has not yet been examined. Bushbuck 55 was inoculated from the same monkey as Calf 33.

There is, of course, the possibility that the extraordinary course of the disease in Calf 33 is due to some immunity peculiar to this individual. Previously, all calves inoculated with this laboratory strain had died of an acute disease.

It will be noticed, however, that the long sojourn in the bushbuck did not render the trypanosome more suited to development in *G. palpalis,* in contrast to the behaviour of *T. gambiense* under similar circumstances.

Trypanosoma uniforme.

As has been pointed out elsewhere, this organism appears to be the most common antelope trypanosome in the Mpumu neighbourhood. According to Bruce and his collaborators, it causes a fatal disease in domestic ruminants, the average duration of the disease in three laboratory-infected goats being 29 days.

My experience of this trypanosome, extending over some 20 months, has not confirmed this opinion. *T. uniforme* has not proved in any way a fatal trypanosome during that time. In only one case has an animal died of an unequivocal *T. uniforme* infection, this being apparently the case with Calf 481, although for months before death no trypanosomes were visible in the peripheral blood.

A characteristic feature of *T. uniforme,* which is especially marked in goats, is the manner in which, after a few weeks, it totally disappears from the peripheral blood as regards ordinary routine examination, while the animal shows no symptoms whatever.

When, however, this trypanosome occurs together with *T. vivax*, or possibly with *T. gambiense*, the animal may become rapidly emaciated, and die with paretic symptoms, particularly in the hind limbs. This, however, is not a constant phenomenon, as is shown in the case of Goat 512, which was infected in November, 1911, simultaneously with *T. uniforme*, *vivax*, and *gambiense*, and at the time of writing is apparently in perfect health.

The following table is of interest, as affording marked contrast to the course of disease described by Bruce:—

Expt.	Infected.	Duration of disease.	Remarks.
Goat 352	Nov., 1910	Killed Dec., 1911	In apparently perfect health at time of death.
„ 397	Jan., 1911	„ „	„ „ „
„ 431	Feb., „	„ „	„ „ „
Calf 481	Jan., „	Died in July, 1912, after 18 months	No trypanosomes visible during last week. Slight emaciation.
„ 536	Feb., „		Killed by leopard. Apparently in excellent health when attacked.
„ 573	Mar., „		Alive and well July, 1912.
„ 616	„ „		„ „
Bushbuck 620	Jan., „		In excellent health July, 1912.
Sheep 534	„ „	Up till date of infection with *T. gambiense* showed good health	Infected with *T. gambiense* in Feb., 1912. Died May, 1912.

In all the above experiments, the strain employed was derived originally from a wild bushbuck shot at the Lake shore.

The strain employed by Bruce and his collaborators was derived originally from oxen, which may explain the difference in the virulence.

Antelope as a Reservoir of T. uniforme.

Two situtunga which were brought alive to the laboratory were found to harbour this trypanosome as a natural infection.

These animals have been kept under observation, and from time to time laboratory-bred *G. palpalis* have been fed upon them. These experiments are summarised in the following table:—

Expt. No.	Period flies fed on Antelope.	Number of flies.				Duration of expt. in days.
		1st day.	30th day.	Dissected.	Containing flagellates.	
464	Oct. 10—18, 1911, on Situtunga 173	57	47	55	0	41
528	Nov. 21—28, „ „ 173	104	84	104	2	55
529	Nov. 22—28, „ „ 173	93	62	93	0	55
706	Apr. 11—17, 1912, on Situtunga 173	86	58	86	3	50
707	Apr. 12—17, „ „ 173	61	47	61	2	49

In all the positive flies of the above experiments trypanosomes were only found in the proboscis.

It is impossible to know when Situtunga 173 became infected, but reckoning from its arrival at the laboratory on June 25, 1911, it will be seen that the animal was still capable of infecting laboratory-bred *G. palpalis* with *T. uniforme* after a period of 10 months.

During this time the animal has remained in excellent health, though confined under by no means ideal conditions.

The question of wild antelope in tsetse regions serving as a trypanosome reservoir thus becomes increasingly important. In this Protectorate alone *T. gambiense, vivax, pecorum,* and *uniforme* have all been proved capable of surviving for a considerable time in these animals, apparently without exerting any injurious effect upon their hosts.

In other parts of Africa *T. rhodesiense, brucei, congolense,* and *nanum* have been recovered from game.

This is not the place for a discussion on the significance of the problem from an administrative point of view. Extermination of the game in Africa is a colossal undertaking, and until something more is known concerning the *rôle* played by birds and reptiles in the spread of trypanosomiasis it would seem inadvisable to set too much faith in this drastic measure.

Note on Merlia normani *and the "Monticuliporas."*

By R. KIRKPATRICK.

(Communicated by Prof. Arthur Dendy, F.R.S. Received August 15, 1912.)

As a result of my recent investigation—carried on with the aid of a grant from the Royal Society—of *Merlia normani*, Kirkp., a siliceous sponge with a supplementary calcareous skeleton, I find that the sponge owes its exceptional character to the fact of its being infested with a Zooxanthella which passes a resting phase in certain cells of the sponge and a motile phase outside those cells.

All degrees of infection can be seen, from those of sponge cells with only a few monads to stages in which the greatly hypertrophied sponge cells are packed with countless numbers of these organisms.

The monads in the resting condition have a cellulose-like coat, and in both phases a nucleus and an orange-coloured chromatophor. In the resting phase many undergo division into 2, 4, 8 and probably more cells.

When masses of sponge cells loaded with monads are examined alive in sea water in a moist chamber, the monads can be seen escaping from their tests and from the sponge cells and swimming about in the water.

In this stage, in which they possess two flagella, conjugation can sometimes be observed between cells of different sizes.

After a period of activity varying from a few minutes to half an hour the motile cells die; but in place of disintegrating, they become calcified.

Well prepared decalcified sections of Merlia show that the calcareous skeleton is made up of bricks, each consisting of a calcified corpse of one of these monads. Possibly under normal conditions many of the monads on their escape from the sponge cells become calcified, and added on to the skeleton without passing through the flagellate stage. Merlia is a lineal descendant of the Palæozoic Monticuliporas, all of which are siliceous sponges with supplementary skeletons formed of the calcified bodies of monads which had lived commensally in the cells of those sponges. The Monticuliporas proper, also species of Chætetes and Rhaphidopora, all contain siliceous spicules of a kind related to those of Merlia, and, further, the calcareous skeleton is formed on the same plan.

The monticules of Monticulipora are the expression of sporadic outbursts of activity on the part of the monads, whereby an extra supply of bricks is formed. Very frequently, *Merlia normani* is found growing over a Melobesia-like crust, which latter directly encrusts the shell or rock. Even in Palæozoic Monticuliporas the same relation holds, for in the case of fossils encrusting shells, a thin graphite-like layer of alga is sometimes found between the Monticulipora and the shell.

At present I am not prepared to say to what genus the Zooxanthella monad of Merlia belongs. I propose to name the species "noronhæ" in honour of Senhor A. C. Noronha, who rendered invaluable assistance during the dredging operations off Madeira and Porto Santo Island. The name Merlia is a synonym of Monticulipora, the name of the sponge being *Monticulipora normani*, Kirkp.

A Camel Trypanosome, with some Remarks on the Biometric Method of Diagnosing Trypanosomes.

By Dr. H. L. Duke.

(Communicated by Sir John Rose Bradford, K.C.M.G., Sec. R.S. Received September 2, 1912.)

The trypanosome which forms the subject of the following experiments was kindly forwarded to Mpumu by Mr. E. Montgomery, M.R.C.V.S., from the veterinary pathological laboratory, Nairobi. The organism was originally obtained from the blood of a camel from Boran. Experiments were undertaken to see whether the trypanosome was transmissible by laboratory-bred *G. palpalis*, and a few sub-inoculations were performed.

Morphology.—Length: 400 trypanosomes taken at random were measured, and the results are given in Table I. As is there shown, the length varies between 18 μ and 34 μ.

Shape: The great majority of the trypanosomes seen are slender; a few forms occur which are markedly broader. The flagellar end may be very much drawn out, the kinetic nucleus being sometimes from 4 μ to 4·5 μ from this extremity.

Undulating membrane: Well developed.

Flagellum: In the slides examined, only one single specimen was observed in which there could be any doubt as to the presence of a free flagellum. In the majority, the free portion of the flagellum is very well marked.

Kinetic nucleus: Always clearly discernible; small and round, situated either almost in contact with the posterior end, or at varying distances from this extremity up to 4·5 μ.

Nucleus: Situated near the middle of the body.

The following Table I gives the measurements from four experimental animals. 100 examples were drawn from each experiment:—

Table I.*

Expt.	18 μ.	19 μ.	20 μ.	21 μ.	22 μ.	23 μ.	24 μ.	25 μ.	26 μ.	27 μ.	28 μ.	29 μ.	30 μ.	31 μ.	32 μ.	33 μ.	34 μ.	Average.
Rat 780............			6	7	11	11	7	6	7	10	8	8	10	6	3			25·1
Rat 696............			1	3	4	7	9	5	5	15	15	9	13	6	5	2	1	27·0
Monkey 646			5	4	8	8	6	11	18	13	16	2	7	2				25·5
Dog 671	1	1	6	6	12	11	11	17	12	15	2	2	4					24·4
Totals	1	1	18	20	35	37	33	39	42	53	41	21	34	14	8	2	1	
Percentages ...	0·2	0·2	4·5	5	8·7	9·2	8·2	9·7	10·5	13·2	10·2	5·2	8·5	3·5	2	0·5	0·2	

* In the table of measurements of the antelope trypanosomes as printed in my paper earlier in this volume (p. 166), two numbers are omitted, viz., in column 18 μ on line (2) 511, monkey, the figure 1 is omitted, and in the line of totals the figure 7 is omitted.

The fly experiments may now be briefly summarised. On no occasion was a successful transmission obtained and no flagellates were found in the flies employed, either in proboscis or gut:—

Table II.

Expt. No.	Period on infected animal.	Number of flies.				Duration of experiment (days).	Result.
		1st day.	30th day.	Dissected.	Containing flagellates.		
680	Mar. 27—30 on Monkey 676	48	35	48	0	33	—
681	,, 28—30 ,, 676	42	29	42	0	32	—
683	,, 31—Apr. 4 on Dog 671	82	56	82	0	30	—
684	Apr. 1—6 ,, 671	47	26	47	0	29	—
687	,, 2—6 ,, 671	62	43	62	0	52	—
693	,, 8—15 ,, 671	79	47	79	0	45	—
698	,, 10—15 on Monkey 676	73	40	73	0	41	—
	Totals	433	276	433	0		

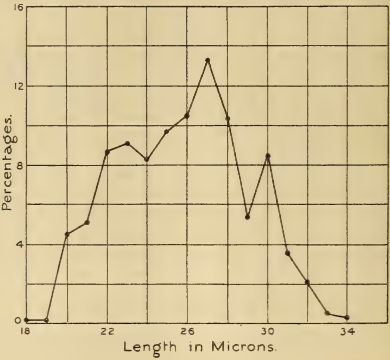
Animals Susceptible to this Trypanosome.—In the following table those experiments marked * were performed by Mr. Montgomery, and I wish to express my indebtedness to him for the permission to quote them. It will be noticed that the disease is rapidly fatal to rats and monkeys, while in dogs it is relatively slow:—

Table III.

Expt. No.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
		days.	days.	
*Ox 123	Camel 8	?	112	Trypanosomes never seen in peripheral blood; proved by subinoculation.
*Mule 6	Dog 3	5	—	Alive after 130 days; not showing by direct observation.
* ,, 25	Donkey 22	?	136	
*Donkey 13	Dog 3	8	99	
* ,, 22	Donkey 13	8	128	
*Dog 3	Camel 8	11	68	
* ,, 5	Ox 123	10	40	
* ,, 6	Dog 3	8	21	
* ,, 14	Mule 6	7	34	
* ,, 21 * ,, 671	,,	?	—	Alive after 150 days; some emaciation; no corneal changes.
* ,, 29	,,	10	62	
* ,, 30	Donkey 22	7	83	
Monkey 676	Dog 671	4	26	Old monkey at commencement of the experiment.
White rat 696	Monkey 676	4	10	
,, 697	,,	4	10	
,, 674	Dog 671	3	10	
,, 675	,,	3	5	
,, 780	Goat 677	?	14	
Goat 677	Dog 671	?	—	Trypanosomes never seen in peripheral blood. Alive and in good condition after 110 days.
Guinea-pig	,,	4	85	
,,	,,	4	70	

Identity of the Trypanosome.—The diagnosis appears to rest between the trypanosomes of the surra-nagana group, *e.g.*, *T. brucei*, *evansi*, *equiperdum*, and *equinum*. The well marked kinetic nucleus seen in stained films excludes *T. equinum*, and the absence of any of the characteristic plaque lesions of dourine is against *T. equiperdum*.

There remain *T. brucei* and *T. evansi* to be considered. The following curve constructed from Table I corresponds roughly to Bruce's curve for *T. evansi*, and the absence of any short stumpy forms from all the experimental animals examined is against *T. brucei*. Thus out of 400 specimens measured in the present instance no trypanosomes were seen measuring less than 18 μ in length, while Bruce describes a considerable number of examples of *T. brucei* between 13 μ and 17 μ. Turning to the table of animal reactions little or no assistance is forthcoming in deciding between *T. brucei* and *T. evansi*. In both species the disease is typically a very rapid one in white rats, and the number of trypanosomes in the peripheral blood shortly before death is enormous. The course of the disease in dogs in the above table is not characteristic of either *T. brucei* or *T. evansi*, which are rapidly fatal to these animals. In the case of surra, however, the disease appears less rapid in its course than with nagana. Thus Laveran quotes experiments of Lingard's in which dogs lived 27½, 29, 34, 36, 47, 97 days after inoculation with *T. evansi*, the last having been inoculated from a naturally infected bovine. In the case of *T. brucei*, however, the same authority gives 26 days as the maximum duration of nagana in dogs.



Though by no means a typical *T. evansi* it is plain that the above experiments suggest this trypanosome rather than *T. brucei*, and this conclusion is supported by the evidence of morphology.

I shall take this opportunity to consider briefly the question of the biometric method of diagnosing trypanosomes from the point of view of a worker dealing with the problem in the field. Of late a great deal of attention has been devoted to this method, with the result that there appears to be some danger of its attaining undue importance. In certain cases it is of undoubted value, as in discriminating between two such species as *T. vivax* and *T. uniforme*, where the difference in size is one of the most important points of distinction. Here, however, it will not be necessary to measure a great number of trypanosomes. In the case of other mammalian trypanosomes, *e.g.*, *T. brucei*, *gambiense*, *pecaudi*, *rhodesiense*, *evansi*, *equiperdum*, *equinum*, *nanum*, and *congolense* exhaustive measurements are of little use in diagnosis.

The following example will illustrate very well both the uses and the limitations of the measurement method. In papers dealing respectively with the morphology of *T. gambiense* and *T. evansi* Bruce gives three curves of *T. brucei* for purposes of comparison. The apices of the curves are at 18 μ, 20 μ, and 24 μ respectively, a very considerable variation. Further, in dealing with *T. evansi*, he claims that from a comparison of the two curves it is possible to distinguish the two species from one another. In the case of *T. brucei* the bulk of the curve will be between 13 μ and 35 μ, with *T. evansi* between 18 μ and 30 μ. A glance at the curves, however, will reveal the fact that the real difference between the two trypanosomes as determined by the biometric method is that *T. evansi* shows no forms of less than 18 μ in length. In other words, if in the course of examining a slide a trypanosome is found below say 15 μ in length, the diagnosis cannot be *T. evansi*. Thus the diagnosis has in reality turned upon the minimum measurements of the two species.

The measurements quoted for the different species of trypanosomes vary with the observer, even although the staining and fixing methods are the same in every case. This variation is due to a number of factors, the least important of which is probably the technique employed in the actual measurement.

It would appear from Miss Robertson's recent work on *T. gambiense** that an endogenous cycle exists in the mammal, the duration of which is variable and incalculable, the result being that the type of trypanosome predominating in the peripheral blood is constantly changing. The date of the infection has no constant connection with the course of the phenomenon. In each cycle the change would appear to be from short to long forms, returning to the short forms again before the temporary disappearance from the blood.

To obtain an accurate conception of the dimensions of any trypanosome it is plain that all the stages of such a cycle must be followed out, from its commencement to its conclusion. Slides taken haphazard from time to time are useless for comparative purposes, as practically any type of curve may be obtained according to the stage of the cycle. The three curves of *T. brucei* quoted above from Bruce's paper are an instance of this. In a recent report summarised in 'Sleeping Sickness Bulletin 36' (vol. 4, p. 145), Dr. Stephens lays great stress on the minute technique of measurement, and points out that Bruce's method is open to criticism. The contention advanced here is that such a criticism, though doubtless true as regards geometrical exactitude, is irrelevant as regards its practical application. The errors involved are so small that they can have no serious effect in the matter of diagnosis, when all the other aspects of the question are considered. Dr. Stephens does not apparently criticise the method of preparing the slide adopted by Bruce, he merely asserts that the actual estimate may err within fractions of a micron. Surely such a source of error cannot be very important in plotting out a curve whose unit is 1 μ, considering, for example, that all values between 5·5 μ and 6·5 μ will necessarily be registered as 6 μ.

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* 'Roy. Soc. Proc.,' B, vol. 85 (No. 582).

Again, there are other considerations which tend to make the biometric method of diagnosis at best inconstant:—

(1) Fixation will vary in different parts of the same slide and in the preparation of slides by different observers.

(2) Numerous varieties of strain exist among trypanosomes of any species.

(3) The great similarity between many so-called species as regards their length variation.

(4) Probably continued maintenance in laboratory animals leads to slight alterations in the morphology of a strain, which to be kept true should from time to time be passed through its insect host. Thus the age of a strain after its last passage through the appropriate intermediate host may be a very important factor in determining its morphology.

In the face of such objections it would appear that, although the biometric method may have a certain value in diagnosis, such refinements as suggested by Dr. Stephens are by no means essential. What is required is that measurements be made in as constant a manner as possible. Dr. Stephens' method, involving as it does a complicated projection apparatus, is quite impossible for workers in the field. This in itself is an objection which would seriously neutralise undoubted advantages. It is, however, difficult to believe that the ultimate results of the application of both methods to the same slide would differ to any appreciable extent. Granting, however, for the sake of argument Dr. Stephens' contention, it must still be remembered that its occasional employment will introduce yet another variable factor into this method of diagnosing trypanosomes.

OBITUARY NOTICE

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SIR JOSEPH DALTON HOOKER. (With three portraits) i

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SIR JOSEPH DALTON HOOKER,* 1817—1911.

Joseph Dalton Hooker, the younger son of Sir William Jackson Hooker, Regius Professor of Botany in the University of Glasgow, afterwards Director of the Royal Botanic Gardens, Kew, and his wife Maria, eldest daughter of Dawson Turner, F.R.S., banker, of Great Yarmouth, Norfolk, was born at Halesworth, Suffolk, June 30, 1817.

Sir William Hooker (*d.* 1865) was himself the younger son of Joseph Hooker, a native of Exeter, where he had been in the employ of Baring Brothers, woolstaplers, with whose family he was distantly connected, and had afterwards gone into business at Norwich. There he married Lydia, daughter of James Vincent, worsted manufacturer, grandfather of George Vincent, the artist. Joseph Hooker was seventh in descent from John Hooker, *alias* Vowell, editor of 'Holinshed's Chronicles,' and uncle of Richard Hooker, the theologian.

At the end of the eighteenth and beginning of the nineteenth centuries the Eastern Counties possessed a rather remarkable amount of local intellectual activity. It developed a notable school of artists, and indirectly fostered the career of many men who attained distinction in various ways. Of this activity Dawson Turner was in some sense a moving spirit. He had himself acquired scientific fame by his botanical work, and he had been the judicious and fortunate collector of a gallery of pictures which have been dispersed, but some of which have found a permanent home in the National Gallery and the Wallace Collection. The facts of heredity are always worth noting, and the descendants of Dawson Turner afford abundant instances. Joseph Hooker was a collector and cultivator of "Succulent Plants." Sir Joseph Hooker thought that his father "presumably derived his love of plants from his father's side, and his artistic powers from his mother's." He, himself, conspicuously inherited both.

In William Hooker's case, Sir Joseph thought that it was a visit to Dawson Turner "which led to the colouring of his future life." He succeeded through the death of a cousin, William Jackson, to a property in Kent, which, had it remained in the family, would have been the source of considerable wealth. Visiting London, he made the acquaintance of Sir Joseph Banks who introduced him into its scientific society. At his suggestion, he visited Iceland, and narrowly escaped death by the burning of the ship on which he was returning. Other prospects of travel in the tropics were frustrated.

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* A fairly complete record of the life of Sir Joseph Hooker could be compiled from the five admirable volumes devoted to the life and correspondence of his father by Dr. Francis Darwin. They have been freely drawn upon. The following abbreviations are used: L.L., 'Life and Letters of Charles Darwin,' 3 vols., 1887; M.L., 'More Letters of Charles Darwin,' 2 vols., 1903; H.L.L., 'Life and Letters of Thomas Henry Huxley,' 2 vols., 1900, which is also quoted.

Without capacity for business he was induced to join Mr. Paget (father of Sir James and Sir George) in a brewery at Halesworth. The venture was unsuccessful; he had sold his property and other "investments were disappointing"; with a wife and four children he found himself crippled by a lavish expenditure on his scientific publications and indulgence in a costly library. He was therefore glad to accept, through the influence of Sir Joseph Banks, the Regius Chair of Botany at Glasgow which had been declined by Robert Brown.

At Glasgow, Joseph Hooker received at the High School the old-fashioned Scotch liberal education; it enabled him throughout life to write Latin with facility. In the University he took the M.D. in 1839, and could recall sitting on the same bench in the old building, now abandoned to a railway company, with Lord Kelvin and Lord Sandford when the father of the latter was Professor of Greek. Some of its teaching left little permanent impression. Thirty years later he said in his Presidential Address at Norwich: "Having been myself a student of Moral Philosophy in a Northern University, I entered on my scientific career full of hopes that Metaphysics would prove a useful mentor, if not a guide in science. I soon, however, found that it availed me nothing."

Hooker, residing under his father's roof, had imbibed from his stimulating intercourse and teaching a passion for botanical research and a keen desire for travel and exploration as a means of extending it. This was gratified when, immediately on taking his degree, he was appointed at the age of 21 to accompany "officially as assistant-surgeon, but in reality as naturalist, the famous expedition of Sir James Clark Ross, fitted out by the Government for the purpose of investigating the phenomena of terrestrial magnetism in the south circumpolar seas." His first actual contribution to scientific literature was a description in 1837 of three new Indian mosses published in his father's "Icones Plantarum." It has been remarked that it is "a curious coincidence" that Darwin, Hooker, and Huxley each "began his scientific career on board one of Her Majesty's ships" (H.L.L., vol. 1, p. 29). It is more than curious when one reflects on the influence on scientific thought which the three men in association were afterwards to effect.

The story of the Darwinian theory unfolds itself like a drama. And there is something fateful in the way in which the three chief protagonists, to whom Sir Charles Lyell must be added, were drawn into its action. In 1839 Darwin published his 'Journal of Researches.' At the Geological Society, where he had "acted as one of the honorary secretaries," he had made Lyell's acquaintance and gave him proof-sheets. These Lyell passed on to his father, Charles Lyell of Kinnordy, a warm friend of the elder Hooker. It is hardly too much to say that this accidental circumstance effected a filiation of the work of Hooker's life to that of Darwin. Hooker tells us (L.L., vol. 2, pp. 19, 20) that the elder Lyell, "taking a kind interest in my projected career as a naturalist, had allowed me to peruse" the proof-sheets. He continues: "At this time I was hurrying on my studies, so as to take my degree before volunteering to accompany Sir James Ross in the Antarctic Expedition, which had just been determined on by the Admiralty; and so pressed for time was I, that I used to sleep with the sheets of the 'Journal' under my pillow, that I might read them between waking and rising. They impressed me profoundly, I might say despairingly, with the variety of acquirements, mental and physical, required in a naturalist who should follow in Darwin's footsteps, while they stimulated me to enthusiasm in the desire to travel and observe." It is important to add that he "received a copy of the 'Journal' complete—a gift from Mr. Lyell—a few days before leaving England."

The Erebus and Terror, "commissioned by Captain Sir James Clark Ross, sailed from Chatham on the 29th of September, 1839." Besides magnetic survey, the collection of "various objects of Natural History" was "enjoined to the officers." Hooker was on board the Erebus with Ross, and it was his good fortune to have a captain whose own tastes were in sympathy with the work; he, indeed, "himself gathered many of the plants" which Hooker subsequently described, and his "private cabin and library were unreservedly placed at his (Hooker's) disposal." The expedition finally gained "the Cape of Good Hope on the 4th of April, 1843, within two days of three years after they had first quitted that port for the high southern latitudes."

On his return, Hooker lost no time in commencing the publication of the botanical results of the expedition. The whole work extended to six quarto volumes, with 2214 pages and 528 plates. The Treasury made a grant-in-aid of £1000 to be expended on the plates; Hooker, for his part, received no remuneration, and abandoned "all share in the proceeds of the undertaking to the publisher, who has thus been able to bring out the series at a much more moderate price than any similar work." The first of the three sections of the whole was devoted to the 'Flora Antarctica' in two volumes (1844–7). To it is prefixed a very matter-of-fact "Summary of the Voyage," which, though it was perilous enough, with a seaman's modesty, lays little stress on the fact. The words, "Both ships had a narrow escape of running foul of an iceberg, over which the sea was breaking, eighty feet high," briefly describe an incident which might have summarily closed Hooker's career. To consummate seamanship must be credited their extrication, and the fact that during the four years of the expedition only two men were lost, one by being washed overboard. There were three breaks in the voyage: one from August 16 to November 12, 1840, in Tasmania; the second, in New Zealand, from August 18 to November 15, 1841; and the third, in the Falkland Islands, from April 6 ("not having seen land for 138 days") to September 6, 1842. These afforded Hooker ample opportunities for making collections, which he afterwards worked out.

When Hooker had completed his 'Flora Antarctica,' he had only reached the age of 30; but it placed him at once in the first rank of systematic botanists. He not merely worked out his own material, but dealt with all that had been obtained from the same area by previous explorers. The result is a classical statement of a problem in geographical distribution which is still far from being completely solved. Writing in 1845, he thought it impossible that the plants of the Antarctic islands could "have migrated from other countries," but also thought "that islands so situated furnish the best materials for a rigid comparison of the effects of geographical position and the various meteorological phenomena on vegetation, and for acquiring a knowledge of the great laws according to which plants are distributed over the face of the globe." This is a somewhat difficult statement. But it appears to imply that distribution followed laws still to be discovered, and the further problem how plants would be modified by isolation due to geographical and physical conditions. In the first he strikes the key-note which was to dominate the whole work of his life; much happened before he saw a satisfactory solution of the second in 1866.

Although the pursuit of a definite aim runs through the bulk of all Hooker's work, it is important to recognise the fact that, like Darwin, he was essentially a naturalist. He was gifted in an extraordinary degree, especially in his early life, with keen powers of observation, a lively interest in what he observed and an aptitude for reflecting upon it. These qualities illuminate his writings, and when he turned aside, as he did from time to time, to some collateral subject, he afforded abundant evidence that, had he pursued it, he would have achieved something more than distinction. The 'Flora Antarctica' affords a striking instance. As Prof. Seward points out ('Fossil Botany,' vol. 1, p. 151), he anticipated the Challenger in discovering "the existence of masses of diatomaceous ooze over a wide area in Antarctic regions" ('Fl. Ant.,' pp. 503–6). Huxley, 40 years afterwards, wrote: "I have always looked upon Hooker's insight into the importance of these things and their skeletons as a remarkable piece of inquiry—anticipative of subsequent deep-sea work" (H.L.L., vol. 2, p. 182).

In 1839 Hooker had been introduced to Darwin in a casual meeting in Trafalgar Square. But Darwin had read with interest the letters sent home to Hooker's family and communicated to him by the elder Lyell (L.L., vol. 2, p. 20). Soon after the return of the expedition, Darwin wrote with warm congratulations, and this began a life-long friendship. What is historically of more moment is that on June 14, 1844, Darwin wrote to Hooker: "I think I have found out the simple way by which species become exquisitely adapted to various ends." "I believe," Hooker tells us, "that I was the first to whom he communicated his then new ideas on the subject." Darwin had long periods of ill-health between this and 1847, but Hooker frequently visited him; "for days and weeks the only visitor, bringing my work with me."

The relations between the two men can have few parallels in scientific history; and we owe a deep debt to Dr. Francis Darwin for the admirable volumes in which they are revealed. It is not easy to say whether the human or the scientific interest is the greater. For Darwin could write in

1856: "How strange, funny, and disgraceful that nearly all our great men are in quarrels in couplets" (M.L., vol. 1, p. 90). With Hooker, the "intimacy," which began in 1843, "ripened into feelings as near to those of reverence for his life-work and character as is reasonable and proper" (L.L., vol. 2, p. 20). Darwin, for his part, could say in 1862: "For years I have looked up to you as the man whose opinion I have valued more on any scientific subject than anyone else in the world" (M.L., vol. 2, p. 284). But it would be a great error to suppose that the terms of the friendship were merely those of mutual admiration. Writing to Lyell in 1866 Darwin said: "His (Hooker's) mind is so acute and critical that I always expect to have a torrent of objections to anything proposed: but he is so candid that he often comes round in a year or two" (M.L., vol. 2, p. 138). The moral elevation of character which made the friendship both possible and profitable is creditable to human nature; but its service to science was deeper. This has been admirably expressed by F. Darwin: "It should not be forgotten that . . . . science owes much to this memorable friendship, since, without Hooker's aid, Darwin's great work would hardly have been carried out on the botanical side; and Sir Joseph did far more than supply knowledge and guidance in technical matters. Darwin owed to him a sympathetic and inspiring comradeship which cheered and refreshed him to the end of his life" (M.L., vol. 1, p. 39).

Darwin and Hooker were both working towards evolution: but they started from different points, and travelled on lines which only slowly converged. At the beginning of the nineteenth century naturalists were still fettered by the chains which Linnæus had forged for them. Species were "diversæ formæ in principio creatæ"; they were permanent and immutable. A variation was "planta mutata a caussa accidentali"; it was not permanent, "reducitur itaque in solo mutato." Species produce individuals which therefore all trace to a common parentage, but do not vary; "produxere plures at sibi semper similes." Darwin tells us that, "when I was on board the 'Beagle,' I believed in the permanence of species" (M.L., vol. 1, p. 367). It was "the relationship between the living and the extinct mammifers in South America" (L.L., vol. 2, p. 34) which first suggested organic evolution to Darwin's mind, in the sense of the replacement in time of one allied organism by another. It was the Galapagos that suggested to him "that the inhabitants of the several islands had descended from each other, undergoing modification in the course of their descent" (Variation, vol. 1, p. 10); this was divergence in space.

Hooker equally started with a belief in the permanence of species. But he was confronted with another difficulty in the presence of the same species in widely dissevered areas. This has been held to be irreconcilable with Linnæus's dictum of a common parentage by writers as recent as the elder Agassiz. Hooker, however ('Fl. Ant.' p. 368), definitely rejected the theory of species having "multiple" or, as he called them, "sporadic" origins. The explanation then must "be sought in some natural cause." For this there are only two alternatives: species must owe their isolation to some means of transport, the view held by Darwin and now largely accepted, though Hooker was long unable to convince himself of its efficiency; or the area occupied by the species must have been once continuous and afterwards dissevered by geological action with consequent climatic change, the explanation accepted by Hooker resting on the support of E. Forbes and Lyell. Darwin himself abandoned Lyell's views and, supported by his own observations, followed Dana in thinking that "continents as continents and oceans as oceans are of immense antiquity" (L.L., vol. 2, p. 76). The trend of recent research is probably on Darwin's side. He was deaf to Hooker's arguments, for, writing to him in 1856, he said, "You cannot imagine how earnestly I wish I could swallow continental extension, but I cannot" (L.L., vol. 2, p. 81). Hooker apparently was never really convinced, for Darwin, writing to Wallace in 1876, congratulates him on his protest "against sinking imaginary continents in a quite reckless manner, as was stated by Forbes, followed alas by Hooker" (L.L., vol. 3, p. 230).

The rejection of the theory of multiple origins seems obvious to us now. But it is important to realise that it was only arrived at cautiously and laboriously. Some years later, Alphonse de Candolle devoted at least half of his great work, 'Géographie Botanique Raisonnée' (1855) to extricating himself from a belief in it. Hooker, in fact, helped to clear the ground for a purely evolutionary theory; for if multiple origins for species are abandoned, the case for their formation by the direct action of the environment collapses as well.

During Hooker's absence on the Erebus a great change had taken place in the position of his father. A botanic garden had been created at Kew in 1759 by the Dowager Princess of Wales, and this had been actively maintained by her son George III for national purposes, with the assistance of Sir Joseph Banks. The mutiny of the Bounty was the outcome of an attempt initiated by him to introduce the bread-fruit of the Pacific into the West Indies. After the death of Banks the fortunes of Kew languished, and, on the accession of Victoria, the Government contemplated its abolition. The proposal met with strenuous opposition, and on the Report of a Committee of Enquiry, Kew as a national botanical centre was reconstituted on a larger scale. The elder Hooker had made Glasgow the seat of botanical work in Great Britain; he had amassed a vast herbarium, and was the possessor of a fine library. These, on accepting in 1841 the post of Director, were transferred to Kew, and on his death were purchased by the Government. But the change, entirely due to public spirit on his part, involved the sacrifice of half his income.

Hooker, therefore, found it necessary to seek some employment. Having acted temporarily, he was a candidate in 1845, with the support of Humboldt and Robert Brown, for the Chair of Botany at Edinburgh. Darwin was despondent at the prospect, but consoled himself with the prophecy which he lived to see vindicated—"I know I shall live to see you the first authority in

Europe on that grand subject, that almost keystone of the laws of creation, Geographical Distribution" (L.L., vol. 1, p. 336). If his unsuccess was a personal disappointment, it was a gain to science, and he was immediately (1846) appointed Botanist to the Geological Survey, in succession to Henfrey.

Hooker records in 1853 that Darwin "directed my earliest studies in the subject of the distribution and variation of species" ('Fl. N. Z.,' p. xxii). One of the first tasks he imposed upon him was the study of the flora of the Galapagos, which was to play so important a part in the development of Darwin's theory. For what it and its fauna impressed upon him was the fact of divergence due to variation and isolation. Hooker made the plants, in 1847, the subject of two papers in the 'Transactions of the Linnean Society.' In the second, he indicates two important principles: the struggle for existence and the effect of isolation, the latter being really a corollary from the former. If the species from the several islands were united in one area, "the strife with its neighbours . . . . would terminate in a few replacing the many" ('Linn. Trans.,' vol. 20, p. 259). Hence it follows that "the first steps towards ensuring the continuance of many species in a given area are to isolate them." As to the crucial problem, the affinity but divergence of the species in the several islands, he contents himself with saying that it "is a mystery which it is my object to portray, but not to explain." That he left to Darwin, whose solution he knew. But that he had some opinion of his own on the subject is evident, as in the same year he instances the Galapagos as a case "where time, the required element for developing such species as are the offspring of variation, has been granted" ('Fl. Ant.,' p. 217).

Hooker's palæontological work was more or less official, and therefore practically limited to the subjects submitted to him. These were discussed in numerous papers up to 1855, after which fossil botany ceased to occupy him. Prof. Seward has kindly examined them in detail, and, surveying them as a whole, finds that "Hooker's contributions to Palæobotany have been the means not only of throwing new light on certain extinct types, but, by their eminently philosophical spirit, of setting a high standard in a subject which has suffered greatly from unscientific treatment at the hands of less cautious contributors, insufficiently trained to appreciate the difficulties of palæobotanical research." In this sense Hooker was a pioneer in the application to such research of a rigid scientific method, which is peculiarly necessary in drawing conclusions from data which are necessarily fragmentary. In three cases he succeeded in establishing new and important facts. The results of an examination (1848) of some remarkable specimens of *Stigmaria*, the root of *Lepidodendron* and *Sigillaria*, were long accepted in text-books. They have, however, since been modified by Williamson's discovery that what Hooker had worked upon was not the actual structure but an inverse cast of it. This, however, did not affect the fact established by him, that the rootlets derive their vascular supply from the main axis. In the same year he threw much light on the morphology and anatomy of *Lepidostrobus*, the strobilus of *Lepidodendron*, and Robert Brown in 1851 records the interesting fact that Hooker had demonstrated to him "the discovery of spores in an admitted species of *Lepidostrobus*." His most masterly achievement was the detection (1854–5) of the true nature of *Trigonocarpon*, a seed-like body not infrequent in the Coal Measures. This is now recognised as the seed of a Pteridosperm. The resemblance which it bears to the seed of *Salisburia* and of Cycads, pointed out by Hooker, has been confirmed by recent research.

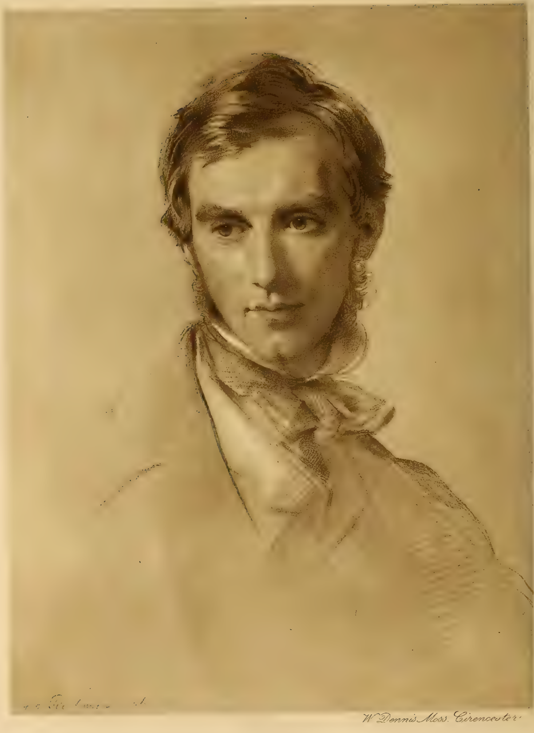
In 1854 Hooker wrote to Darwin, "From my earliest childhood I nourished and cherished the desire to make a creditable journey in a new country, and write such a respectable account of its natural features as should give me a niche amongst the scientific explorers of the globe I inhabit, and hand my name down as a useful contributor of original matter" (M.L., vol. 1, p. 70). It must seldom happen that the wish of childhood is so completely and admirably realised in after life. It was a fortunate circumstance that the Earl of Carlisle was Chief Commissioner of the Department of Woods and Forests, to which both Kew and the Geological Survey were attached, and that both the Hookers were known to him. Dr. Falconer, Superintendent of the Calcutta Botanic Garden, had suggested the exploration of the Central and Eastern Himalayas, and Humboldt warmly supported it. Lord Carlisle, on the ground of securing the prospective collections for Kew, obtained Hooker a grant from the Treasury of £400 per annum for two years, and the expedition assumed the character of a Government mission. Lord Auckland, the First Lord of the Admiralty, wished him after India to go to Borneo at the Government expense "for the purpose of reporting on the capabilities of Labuan," and he received a commission in the Navy. With Lord Auckland's death the project fell through, but he was allowed £300 for a third year in India. The Admiralty sent him out on H.M.S. Sidon, which conveyed to Egypt Lord Dalhousie, the Governor-General of India, who from this point attached Hooker to his suite and subsequently procured him admission into Sikkim. While resident there he was for some time the guest of Brian Hodgson, the Buddhist scholar and naturalist, to whom he was indebted for much assistance and information. Hooker, with Dr. Campbell, the Governor-General's agent, was imprisoned by the Sikkim Court in the hope of bringing some influence to bear in a dispute with the Indian Government; but he fortunately escaped the ill-treatment to which his friend was subjected. He was able to explore part of Eastern Nepal in which no traveller has since succeeded in following him.

The expedition was rich in results, and not least remarkable for the versatility which Hooker displayed in obtaining them. He surveyed the passes into Tibet, a task of no small difficulty, seeing that it was accomplished single-handed and in a mountainous region of extreme complexity. Later travellers have only confirmed the accuracy of his work. The Lhasa Mission in 1903 derived such aid from its use that, having reached Khambajong, where it was no longer available, Sir Frank Younghusband sent Hooker a congratulatory telegram. This, with pardonable pride, he hung framed in his dining-room. Hooker's geological observations were the first (as they are still a principal) source of our knowledge of the physical structure of Sikkim. He had had " a very extensive experience of ice in the Antarctic Ocean," and in the Himalayas he was confronted with glacial phenomena on the largest scale. He thought that " very few of our geologists appreciate the power of ice as a mechanical agent " ('Him. Journ.,' vol. 2, p. 121). He gives a clear explanation of the terracing of mountain valleys, like the " Parallel Roads of Glen Roy," as the beaches of glacial lakes. He appears to have sent this home to Darwin with some expectation of publication. Both he and Lyell thought the " evidence ought to have been given more distinctly " (L.L., vol. 1, p. 376). But at this time Darwin held the view, which Lyell adopted, that the Glen Roy terraces were the result of marine action. Hooker seems to have felt discouraged, and spoke " of giving up Geology " (M.L., vol. 2, p. 152). But twelve years later (1861) Darwin had abandoned his early theory and wrote significantly to Hooker, " It is, I believe, true that Glen Roy shelves (I remember your Indian letter) were formed by glacial lakes " (M.L., vol. 2, p. 190). Hooker by continued and laborious observations laid the basis of the meteorology of Sikkim. He succeeded in introducing into cultivation through Kew its splendid Rhododendrons, which were worthily illustrated from his drawings in a work edited by his father (1849–51) and published during his absence. 1850 was spent in travelling with his old Glasgow class-mate, T. Thomson, in Eastern Bengal and the Khasia Hills, returning to England the following year. A vast collection of some 6000 to 7000 species of plants represented by copious duplicates was brought back. The Treasury made him a grant of £400 annually for three years to name and distribute his specimens (some sixty herbaria in Europe, India, and the United States were recipients), and to write the 'Himalayan Journals,' which appeared in 1854, and were dedicated to Darwin; never, probably, were the results of an expedition dealt with so swiftly or so rapidly made available for work of permanent scientific value. Darwin declared it to be " a first-class book." Douglas Freshfield, in dedicating to Hooker (1903) his own, describes him as " the pioneer of mountain travel in the Eastern Himalaya," and as " still our chief authority on Sikkim." It is singular how few have been tempted to follow his footsteps. They might have been so, for Freshfield writes, " The only European who had stood on Chungjerma before me, Sir Joseph Hooker, has described the scenery and the effects of atmosphere he witnessed on the road in what is perhaps the most eloquent passage in his admirable volumes " (p. 196).

In 1855 Hooker published 'Illustrations of Himalayan Plants,' from drawings at Kew, made at Darjeeling, at the expense of J. F. Cathcart, including *Hodgsonia*, the gigantic Cucurbit which he dedicated to his friend Hodgson. He had now been able to resume work on his Antarctic collections, and (1853–5) published the 'Flora Novæ-Zelandiæ,' forming the second instalment of the 'Botany of the Antarctic Voyage.' On its completion the Government of New Zealand awarded him an honorarium of £350, and subsequently commissioned him to reproduce it in the more convenient 'Handbook' (1864–7).

Huxley has stated that "the facts of variability, of the struggle for existence, of adaptation to conditions, were notorious enough" anterior to the publication of the 'Origin' (L.L., vol. 2, p. 107). This is true enough as to the second and third, but by no means the case as regards the first. In 1852 Herbert Spencer had grasped the principle of the "struggle for existence," and was always surprised that he had not also deduced from it the "obvious corollary" of natural selection, or, as he called it, "survival of the fittest." But he found an explanation of his not having done so in the fact "that I knew little or nothing about the phenomena of variation" ('Autobiogr.,' vol. 1, p. 390). Nor is this to be wondered at; Linnæus had ignored it, and Hooker in the Introductory Essay to the 'Flora Novæ-Zelandiæ' (p. 8) could say, in 1883, "I am not acquainted with a British or Continental Flora which attempts to give a general view of the variation and distribution of the species described in it." We owe it entirely to Hooker's precept and example that no work of the kind is published now without it.

Variation being the indispensable material for natural selection to work upon, it is not surprising that Darwin had early suggested its study to Hooker. And though Darwin could say in 1857, "What a splendid discussion you could write on the whole subject of variation" (L.L., vol. 2, p. 90), the Introductory Essay and the application of its principles in the Flora itself, broke down once for all the Linnean principle of specific invariability. Hooker showed that it had only been maintained at all by treating "every minute character" as of specific value; the standard of specific distinction would therefore widely differ even in allied genera. Hooker's "plan," which marks a turning point in descriptive botany, was to give specific characters "the same relative value." The result would be that, while some species would be sharply defined, others would be an assemblage of closely allied forms. He saw that variation was of two kinds: one which was spontaneous and not explicable; the other, due to the direct action of external causes. As regards the first, he thought "we must ultimately adopt much larger views of the variation of species than heretofore," but was not prepared to admit that it would "obliterate specific character." As regards the latter, he thought that "climatic differences . . . often induce change"; that "a sufficient time may isolate" them; and that "such races frequently retain their character even when they have been under cultivation for many years." But he points out that the argument in favour of species being created from the fact that they are often "nicely adapted" to climate is vitiated by the evidence of geographical distribution, that they frequently exist under widely dissimilar climatic conditions. There is no trace here of any recognition of the part played by natural selection. Darwin pointed out that no theory as as to the "origin of species" makes "any difference in descriptive work"



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(M.L., vol. 1, p. 453). Though striving to arrange species according to their affinity, it deals with them as existing facts. Hooker therefore proceeds on the assumption that species are permanent, but guards himself by saying that this was not to be "interpreted . . . . as a fixed and unalterable opinion."

In reviewing the facts of distribution in the Southern Hemisphere, he insists on the inefficiency of known means of transport for seeds, and expresses the belief, which he had only hinted at before, that the presence of the same species in separate Antarctic islands could only be explained by their having been parts of a continuous area now partially submerged. He extends this theory to explain the presence of a South American element in the New Zealand flora.

In 1855 Hooker was appointed to the Kew Staff as Assistant Director, and for the next thirty years of his life he remained attached to it. With T. Thomson he had commenced a 'Flora Indica,' and the first and only volume was published in this year. But it was projected on too vast a scale to be practicable, and the work was only accomplished when resumed in later life on a more restricted plan. The first attempt was not, however, fruitless. It included an "Introductory Essay," which was published separately. This contains a "Summary of Labours of Indian Botanists," which is still indispensable, and a masterly "Sketch of the Physical Features and Vegetation of the Provinces of India," which required substantially little modification at his hands fifty years later, when the whole flora had been systematically worked out.

The great problem which never ceased to occupy Hooker's mind from his earliest work on the Antarctic flora was "the laws of the distribution of plants." and he approached it by a purely inductive method. The ascertainment of the facts must precede any attempt to theorise about them. He would give no countenance to "loose theories on geographical distribution and on the development of species" (Intr. Ess., 'Fl. Ind.,' p. 102). Though acquainted with the progress of Darwin's speculations and constantly assisting him with information, his attitude was one of continual criticism and reserve. Darwin would accuse himself of "mere base subservience and terror of Hooker and Co." (L.L., vol. 2, p. 335). "Adios," he wrote to Hooker in 1858, "you terrible worrier of poor theorists" (M.L., vol. 1, p. 105). Darwin's theory must fit in with geographical distribution, and Darwin agreed that that would be a test (L.L., vol. 2, p. 78). Meanwhile, Hooker more than once insists "that progress in this branch of botany depends on an exact knowledge of species, genera, and families, and their affinities" (*loc. cit.*, p. 103), and to this he henceforth devoted himself.

It is to be remarked that in this essay he expresses the opinion that the principle of the struggle for existence amongst plants, the first enunciation of which he attributes to Dean Herbert, had "never been sufficiently appreciated" (p. 41). "Species in general do not grow where they like best, but where they can best find room." The facts of Indian vegetation led him to anticipate œcology, the study of plants in their social relations, which is a modern outcome of the Darwinian theory. He notes that he found some forty species on a "tree-stump on the damp, exposed hill-tops of the Khasia." He continues: "It is almost impossible, however, to appreciate the nicely balanced local circumstances that determine the number of species which will all find room and keep, and in a limited space" (p. 92). It was on such cases that Darwin founded one of the strongest arguments, if a somewhat subtle one, for his theory. "The truth of the principle that the greatest amount of life can be supported by great diversification of structure is seen under many natural circumstances ('Origin,' 6th Edition, p. 88).

Besides permanent and transitory variation, he now recognises a third class: "There are accidental variations due to no apparent causes or to very fluctuating ones" (p. 30). This is probably the first use of the word fluctuating in connection with the kind of variation to which it is now generally applied.

There was a gradual approximation to Darwin's own point of view. Huxley (L.L., vol. 2, p. 196) quotes a letter from Lyell to Sir Charles Bunbury, written April 30, 1856: "When Huxley, Hooker, and Wollaston were at Darwin's last week, they (all four of them) ran atilt against species—further, I believe, than they are prepared to go." Huxley adds: "With regard to Hooker, he was already like Voltaire's Habbakuk, *capable de tout* in the way of advocating evolution." But a belief in evolution as a fact, and a conception of how it is effected, are by no means identical. He was still some way from accepting Darwin's solution.

It is probably unique in the history of science for the author of a far-reaching theory, which ultimately meets with general acceptance, to keep it for twenty years as a secret to himself and to a few intimate friends, and meanwhile to occupy himself in testing its validity in every possible way, and in building up argument in its support. Such reserve would in any ordinary case be perilous if the claims of priority were of any importance. But Darwin found that, as soon as the necessary consequences of the theory were appreciated, the opposition it would encounter would be so violent that no pains would be thrown away in making the evidence in its favour as far as possible unassailable.

Lyell had warned him that he would be forestalled. On June 18, 1858, he received from Wallace, who was then in the Celebes Islands, an essay containing what was substantially his own theory, and it came upon him, as Wallace said, "like a thunderbolt from a clear sky." The position became tragic, for, on June 29, Darwin was prostrate with illness; scarlet fever was raging in his family, and an infant son had died of it the day before. Lyell and Hooker acted for him; an extract from the MS., shown to and read by Hooker in 1844, was communicated with Wallace's essay to a meeting of the Linnean Society on July 1. This itself was an exceptional occasion, for it was a special and postponed meeting in consequence of the death of Robert Brown. The new theory was launched over the grave of the most distinguished botanist of his time.

Darwin was forced to what, according to Wallace, "he considered a premature publicity." This, in the interests of science, was perhaps not an unmixed evil. For, with feeble health, it may be doubted if he would ever have achieved the series of works in which he intended to promulgate it. He was obliged immediately to prepare an abstract, the 'Origin of Species,' which appeared in 1859. This had, at any rate, the advantage that it gave the theory to the world with a summary, in the most concentrated form, of the facts and arguments which seemed to the author to support it.

But it had another effect. Darwin's friends, who had been in his confidence, had now to decide one way or the other. Hooker gave a general adhesion at once, though with some reserve. He would doubtless have done so earlier, if to do so would not have been to force Darwin's hand. Writing to Hooker on Christmas Day, 1859, he said, "I do think I did you a bad turn by getting you to read the old MS., as it must have checked your own original thoughts" (L.L., vol. 2, p. 252). This has since been published (1909) by Francis Darwin in 'The Foundations of the Origin of Species.' In August, 1854, in case this abstract might prove, in the event of his death, to be the only record of his work, he had made a note: "Hooker by far best man to edit my species volume" ('Foundations,' p. 28). The fact that Hooker had read it as early as 1844, when it was written, saved the situation. There is no evidence that a knowledge of its contents hampered his own progress to evolutionary theory. He had to clear his path of obstacles which were peculiar to his own subject; difficult as it may be to appreciate the fact now, the doctrine of "multiple origins" hung round the neck of botanists like a millstone. A result of its avowed or tacit acceptance was, to use Hooker's words, "the too prevalent idea that the plants of newly discovered, isolated, or little-visited localities must necessarily be new." The resulting incubus of synonymy he described as "the greatest obstacle to the progress of systematic botany;" and he gave copious illustrations from the Indian flora. The evil can only be remedied by comparing new material in large herbaria; in no other way can the geographical distribution of a species be arrived at.

In 1859, however, Darwin was able to write to Wallace: "Hooker, who is our best British botanist, and perhaps the best in the world, is a full convert, and is now going immediately to publish his confession of faith" (M.L., vol. 1, p. 119). But, writing to Hooker, he said: "I have spoken of you . . . . as a convert made by me; but I know well how much larger the share has been of your own self-thought" (L.L., vol. 2, p. 176). The conversion must have been fairly rapid at last, for Darwin had written to him the year before "not to pronounce too strongly against natural selection" (L.L., vol. 2, p. 138).

The 'Flora Tasmaniæ,' completing the 'Botany of the Antarctic Expedition,' was published in parts between 1855 and 1860. To the first volume, issued in 1859, is prefixed an introductory essay, "On the Flora of Australia: its origin, affinities, and distribution." This is by far the most important and remarkable of all Hooker's speculative writings. Published in June, it anticipated by four months the appearance of the 'Origin of Species,' to which it served as a most efficient advanced guard.

He avoids any precipitate advocacy, and takes a purely judicial view—"matured conclusions on these subjects are very slowly developed." "I have hitherto," he says, "endeavoured to keep my ideas upon variation in subjection to the hypothesis of species being immutable," as a check "to careless observation of minute facts." He finds "the aspect of the question materially changed," and proceeds "to review, without reference to my previous conclusions, the impression which I have derived from the retrospect of twenty years' study of plants." He points out that he had long insisted on the importance and prevalence of variability, and "how deep it lies beneath the foundation of all our facts and reasonings concerning classification and distribution." He now saw that the "mutual relations" between species were "analogous to those between the lineally descended members of a family," and that "this indeed is the leading idea in all natural systems." Both distribution and classification had unconsciously led botanists towards evolution. When it was once grasped that similarity in species implied a common ancestor, it became possible to form some theory to explain the presence in any one country of elements characteristic of others though not identical.

The essay contains a masterly analysis of the Australian flora. Seven-eighths of its species were found to be entirely confined to it. Bentham four years afterwards described it as "the best exposition I am acquainted with of the geographical relations of the flora of any country." When he had completed in 1878 his own 'Flora Australiensis,' in which he described the species in detail, he found no ground for modifying Hooker's conclusions. On the completion of the work Hooker received an honorarium of £350 from the Tasmanian Government.

The 'Origin of Species' soon removed from Hooker's mind any doubt as to the efficiency of natural selection. Darwin wrote to Hooker in 1862 to say that "my present work is leading me to believe rather more in the direct action of physical conditions. I presume I regret it, because it lessens the glory of natural selection" (M.L., vol. 1, p. 214). Hooker, however, was now eager to exalt it. Writing to Bates ('River Amazons,' p. 43) he said, "I am sure, with you as with me, the more you think the less occasion you will see for anything but time and natural selection to effect change; and that this view is the simplest and clearest in the present state of science." Darwin remarked on the correspondence, "It is really satisfactory to me to see so able a man as Bates (and yourself) believing more fully in natural selection than I think I even do myself" (M.L., vol. 1, p. 199). Hooker, however, thought that variation was paramount and, in writing to Bates, "enough with time to beget any amount of change," and he adds: "Variation I hold to be centrifugal; if it were not so, how could it go on making species, which are only the preserved forms of each brood which circumstances favoured?" On this Darwin commented: "I rather demur to your doctrine of centrifugal variation" (M.L., vol. 1, p. 199), and seemed to think that it conflicted with his "Doctrine of Diversification." This is the principle already mentioned, that the greatest amount of life can be supported by great diversification of structure ('Origin,' 6th Ed., p. 88). Hooker's own observations in the Khasia had taught him this. His own point was different, and the explanation he gives, "the best marked varieties of a wild species occurring on the confines of the area the species inhabits," is undoubtedly the fact that proves the point. Both Hooker and Darwin rejected the popular belief that species which had varied could revert to the original type. In other words, evolution is not a reversible process, as Hooker clearly saw. F. Darwin in discussing the point observes that this does not conflict with Galton's "Regression to Mediocrity," which is a "centripetal tendency." For that only applies to a population which interbreeds freely, where the amount of variation always regresses to what Galton terms the median value. Such regression tends to wipe out variability and to establish racial stability. But the median is itself subject to variation and to natural selection.

The essay on the Australian flora has so far been dealt with only as an apologia for Hooker's position with regard to Darwinian theory. As was rightly stated on the occasion of the award to him of the Copley Medal, "it effected a revolution" in respect to the rational basis on which he placed geographical botany. De Candolle in his monumental work, 'Géographie Botanique Raisonnée,' published in 1855, only four years before the 'Origin,' left the problem unsolved. Asa Gray remarks truly, "De Candolle's great work closed one epoch in the history of the subject, and Hooker's name is the first that appears in the ensuing one."

In 1881 Hooker made Geographical Distribution the subject of his address as President of the Geographical Section at the Jubilee meeting of the British Association at York. He showed that from Linnæus onwards the distribution of plants was regarded as dependent solely on physical conditions. Meyen, for example, in his 'Geography of Plants' (1836), of which the Ray Society published a translation in 1846, lays down that "conditions of climate, particularly heat and moisture, are the chief causes which determine the station and distribution of plants" (p. 8). Hooker in this address pointed out that such conclusions failed to give any explanation of the occurrence of similar organisms "when there is no discoverable similarity of physical conditions, and of their not occurring in places where the conditions are similar" (p. 7). Dependence on physical features was still, however, maintained by Grisebach in 1872.

In the concluding pages of the essay on the Australian flora Hooker briefly states the general conclusions at which he had arrived as to the actual facts of plant distribution and of their origin in the past. These have become classical and the basis of all subsequent speculation. The "general indications," which, as Bentham pointed out, we owe to Hooker, were amplified by himself into a more detailed survey in his 1869 address to the Linnean Society. Hooker insisted on the distinctness of the two great Northern and Southern floras. While admitting that they may have had a common origin in the past, it could not have been "within comparatively modern geological epochs." He puts them in striking contrast. The Northern occupies a vast land surface from which it has sent down invading streams in every direction southwards; its "tendencies" are "usurping." The Southern, on the other hand, is broken up into three subordinate floras in dissevered areas; its northward migration, if it be one, and not as Darwin thought a retreat, is feeble. It appears to be doomed to extinction, and everywhere we see its peculiar forms "dying out in small areas." They are subject in fact to the nemesis of excessive specialisation, which implies a greater antiquity. They will succumb before "that power of appropriation in the strife for place" in which Hooker saw a "force . . . of the real nature of which power no conception has been formed by naturalists, and which has not even a name in the language of biology" (p. civ). Hooker singled out as notable the "continuous current of vegetation" which extends from Scandinavia to Tasmania, "the greatest continuity of land" "of the terrestrial sphere," and the next in importance the Himalayan along the same arc, dying out in Malaya. The former he worked out (1862) in great detail in his classical memoir, "Outlines of the Distribution of Arctic Plants" ('Trans. Linn. Soc.,' vol. 23, pp. 251–348). He showed that "the Scandinavian vegetation . . . in every longitude . . . migrated across the tropics of Asia and America" (p. 253). Few now probably will accept Darwin's explanation that this took place during a refrigeration of the tropics; the facts established by Hooker remain, however, unshaken. Darwin in this and other cases sometimes indulged in hazardous deductive speculation; Hooker relied on laborious inductive investigation. The result was to place plant distribution on an entirely new basis. The flora of a country could no longer be regarded as the outcome of local physical conditions, but was derivative from a former order stretching back into a remote past. The gulf between the two conceptions is immeasurable.

In 1866 (August 27) Hooker delivered before the British Association at Nottingham his celebrated lecture "On Insular Floras." This was published at the time in the 'Gardeners' Chronicle,' but not separately printed (without alteration) till 1896. He indulged in an amusing allegory to represent the celebrated discussions at Oxford (L.L., vol. 3, p. 48) in which he had been called on to take part, and had triumphed over the Bishop of Oxford's ignorance "of the elements of botanical science" (L.L., vol. 2, pp. 322–3). He thought that "neither geological considerations, nor botanical affinity, nor natural selection, nor all these combined, have yet helped us to a complete solution of this problem, which is at present the *bête noire* of botanists" (p. xv). He concluded that "the hypothesis of trans-oceanic migration, though it leaves a multitude of facts unexplained, offers a rational explanation of many of the most puzzling phenomena that oceanic islands present—phenomena which under the hypothesis of intermediate continents are barren facts" (p. 33). Darwin thought the arguments for occasional transport were given with perfect fairness and would receive a fair show of attention as coming from a professed botanist (L.L., vol. 3, p. 48).

In the essay on the flora of Australia, Hooker pointed out that "there is a strict analogy . . . between the floras of islands and those of lofty mountain ranges" (p. xv). He then thought that the "species and genera common to . . . distant localities" could only be explained by "conditions which no longer exist." The subject long continued to interest him. In papers in the 'Journ. Linn. Soc.' he discussed (1862) the plants of Clarence Park, 10,000 feet, and of the Cameroons (1864), 13,000 feet. In the appendix to 'Morocco and the Great Atlas' (1874) he summarised the results. This was the first study of the vegetation of any African mountains outside Abyssinia. He ascertained the remarkable fact of "the preponderance of Abyssinian genera and species"; this was "proved by almost all of the genera and half the species being natives of Abyssinia," 2000 miles distant. Darwin thought this a "wonderful case," and confirmation of his belief "that the whole world was cooler during the Glacial Period" (M.L., vol. 1, p. 477). In 1874 he similarly discussed the first collection made on Kilimanjaro. This was found to be mainly South African in affinity, but with species common to the Cameroons on the one hand and Abyssinia on the other. Thus was again established a connection at high levels across the tropics, but in the reverse direction to that of the Scandinavian, of the great Southern and Northern temperate floras. It is noteworthy that in 1847 Hooker had been sceptical as to the existence of mountains in Central Africa at all ('Ant. Fl.,' p. 210). It was to Robert Brown that the first detection of a South African element in Abyssinia was due (*loc. cit.*).

Hooker had now played his part, and felt that he had played it, in the great campaign. He wrote to Darwin in October, 1859, to say that he now intended "sticking to humdrum science." The intention was deliberate, though Darwin ridiculed it as impossible. Hooker had been appointed Assistant Director at Kew as a lieutenant to his father, to whom he was deeply attached. He would certainly stand at his right hand during his lifetime, and there was probability amounting to certainty that he would be called upon to succeed him. His lot was therefore cast in with Kew, and this he saw clearly involved scientific duties and responsibilities which Darwin imperfectly apprehended. Kew had been organised both as the national headquarters of botanical investigation and as a consultative department of the Government in colonial enterprise. If the two functions are correlative, the latter may sometimes handicap severely the former.

The central feature of the Kew organisation is the vast herbarium, of which the foundations were the unsurpassed collections accumulated by Sir William Hooker, and purchased by the Government after his death. The elder Hooker had always held consistently that a herbarium was essentially an instrument of research, and that a vital activity was a necessary condition of its usefulness. The continuance of this policy has led to the enormous development of the Kew Herbarium by bequests and the continuous influx of collections made by travellers and explorers. The problem which had for some time weighed on Hooker's mind was how to improve the classification of this vast amount of material and throw it into a form more available for detailed research.

For the preceding two centuries botanists had seen that the first step in a sound classification of the vegetable kingdom was to gather species into genera. The difficulty was to discard characters which were superficial and to decide on those which correctly indicated a true affinity. The task commenced by Linnæus had been in a great measure accomplished by the logical spirit and method of the French school. In the early part of the last century Endlicher had attempted to review the whole field with indifferent success. Hooker and Thomson (" Introd. Ess.," 'Fl. Ind.,' p. 10) could only lament that "so eminent a botanist . . . . has thought it necessary to encumber his pages with characters of genera which must for ever remain enigmatical, unless some happy chance should make us acquainted with the specimens of the authors." Hooker felt that this sort of compilation at second hand was worthless for any scientific end. A further and no less serious defect in current taxonomy was the absence of any uniform standard. "A knowledge of the relative importance of characters can only be acquired by long study; and, without a due appreciation of their value, no natural group can be defined. Hence, many of the new genera which are daily added to our lists rest upon trivial characters, and have no equality with those already in existence." This may be taken to heart by those who are disposed to estimate lightly the value of taxonomic work. Its successful prosecution depends not merely on an insight into morphology, but still more on a judicial power of co-ordinating evidence. John Stuart Mill bears eloquent testimony to its value as a discipline in this respect. Jeremy Bentham does no less, and it is scarcely too much to say that a study of the methods of "classification in natural history" stimulated him in the pursuit of an ideal jurisprudence.

In 1860, Hooker commenced with his friend George Bentham (Jeremy Bentham's nephew) the 'Genera Plantarum,' and it occupied them continuously for the succeeding quarter of a century. The first portion was issued in 1862, the concluding in 1883, Bentham only surviving its completion till the following year. The two worked together, but the major part of the task was borne by Bentham, who, having independent means, was not subject to the official and other calls upon his time which hampered Hooker.. It is to be noted that the work was written throughout in the Latin language, and the title, 'Genera Plantarum ad exemplaria imprimis in Herbariis Kewensibus servata definita,' establishes the fact that it is entirely based on material which is open to any subsequent investigator for verification.

Something must be said as to the individual qualities of the partners in this memorable achievement. Bentham, trained in the French school, brought to the work those elements of form, precision, and logical method which have never been surpassed. To this he added, in the words of Prof. Oliver, "an insight of so special a character as to deserve the name of genius, into the relative value of characters for practical systematic work, and, as a consequence of this, a sure sifting of essentials in each respective grade." Hooker's strong point, on the other hand, was a keen appreciation of the value of morphological characters as a guide to affinity. Reichenbach found in his work "that touch of genius which resolves difficult questions of affinity where laborious research has often yielded but an uncertain result" (Copley Award). Hooker's share, therefore, exhibits more originality, and he felt some disappointment that this had not received the recognition it undoubtedly deserved.

Darwin at first could not "help being rather sorry at the length of time it must take" (M.L., vol. 2, p. 281), but twenty years later thought it "a great misfortune for science" that Hooker could not devote more to it (M.L., vol. 2, p. 433). Its merits found universal recognition. It was described abroad as a work worthy of German laboriousness and of more than German accuracy. Perhaps the most signal estimate of its value is that it has been freely drawn upon by every succeeding writer in the same line. Incidentally, it is a mine of information on Geographical Distribution which has never yet been utilised. The area occupied by each genus is carefully worked out. Casimir de Candolle has made some attempt to tabulate the data, but merely states verbally the conclusion that the origin of existing phanerogamic vegetation was intertropical. It appears from a letter of Darwin's in 1870 (M.L., vol. 1, p. 323) that at that time Hooker contemplated "some general work on Geographical Distribution," and it is an irreparable loss to science that he was never able to give effect to his intention.

In 1873 he edited 'A General System of Botany,' a translation by his first wife from the 'Traité Général' of Le Maout and Decaisne, in which he rearranged the Dicotyledonous orders according to the sequence adopted by Bentham and himself. For the Monocotyledons he devised a new classification of his own.

In 1861 Henslow, Hooker's father-in-law, and Darwin's "dear old master in Natural History" (L.L., vol. 2, p. 217), died. The reverence that is compatible with the keenest scientific criticism of belief may be measured by the noble letter which Huxley wrote to Hooker. A sentence may be quoted: "I can faintly picture to myself the great and irreparable vacuity in a family circle caused by the vanishing out of it of such a man as Henslow, with great acquirements, and that great catholic judgment and sense which always seemed to me more prominent in him than any man I knew" (H.L.L., vol. 1, p. 226).

On November 3, 1864, the *x* Club was started. It consisted of nine scientific men, all intimate friends, but whose occupations gave them otherwise little opportunity of keeping in touch. The arrangement was to dine on a Thursday in each month on which the Royal Society met. Three of the members were destined to be Presidents in succession, and three Copley medallists. It was the opinion of the smoking room of the Athenæum that "they govern scientific affairs, and really, on the whole, they don't do it badly." Huxley repudiated this, and thought the "tone of our ordinary conversation" was "sadly frivolous" (H.L.L., vol. 1, p. 259). There was doubtless a method in the frivolity. Anyhow the club was a power, and the nine formed a sort of dynasty while it lasted; and dynastic vigour has its merits. It died out in 1893. Hooker, as well as Huxley, was also a member of "The Club," and occasional attendance at its dinners was in later life one of the few social pleasures he allowed himself (H.L.L., vol. 1, p. 259).

He had never lost his interest in glacial phenomena. July of 1865 was spent in Teesdale, and the result was a paper on 'The Moraines of the Tees Valley.' On August 12 his father died. The circumstances were tragic: the illness was sudden; Hooker sent to London for the best advice, but to no purpose. He relates, "I saw him no more, for, sleeping on the floor by his bedside that night under an open window, I was suddenly prostrated with rheumatic fever." Hooker succeeded to the directorship, and for the next twenty years administrative duties of the most varied kind limited still more the time available for scientific work. Official scientific positions in this country are apt to be regarded as sinecures; perhaps this is because their occupants often achieve as much as more leisured men.

The elder Hooker had carried the Government with him in a wave of enthusiasm in reorganising Kew on a scale worthy of a national establishment. It was not, therefore, to be expected that Hooker's directorship would be signalised by any great undertakings of equal magnitude. Much, however, was done in detailed development, and some notable features were added. In 1869 the "New Range" replaced eight obsolete "stoves," and in 1877 a new wing relieved the intolerable congestion of the Herbarium. Various additions were the result of liberality independent of the Government. The Jodrell Laboratory was built (1876) at the expense of T. J. Phillips Jodrell, to give effect to a recommendation of the Commission on Scientific Instruction and the Advancement of Science "that opportunities should be afforded for the pursuit of investigations in Physiological Botany." It has amply fulfilled its purpose, and has been the scene of much memorable work. The North Gallery, completed in 1880, was erected at the expense of Miss Marianne North, to contain the pictures which she had made of tropical vegetation in various parts of the world. In the following year an extension was made to the Museum, at the expense of the India Office, to allow space for the botanical collections removed from the Indian Museum, and the Rock Garden was formed to receive the collection of Alpine plants bequeathed by George Curling Joad, F.L.S. A hailstorm on August 3, 1879, was the greatest trial to which Kew has been subjected; 38,649 panes were smashed into 18 tons of broken glass, and the damage required for its repair a supplementary estimate in Parliament. In 1876 the introduction of the Para rubber tree into our Eastern Colonies was successfully accomplished, though not without considerable difficulty. A quarter of a century elapsed before it was seen to contain the "potentiality of growing rich beyond the dreams of avarice."

Hooker's personal hobby was the development and extension of the Arboretum, or collection of ligneous plants grown in the open air. This had been commenced by his father, and he spared no pains in enlisting the aid of correspondents at home and abroad in enriching it. Nor was he less anxious to have them correctly named, and the often deplorable confusion in their nomenclature cleared up. Elwes and Henry, in their great work, 'The Trees of Great Britain and Ireland,' have reaped a rich harvest from Hooker's labours. He finished planting the Pinetum in 1870; there was no part of the establishment which he revisited with more pleasure after his retirement. The Arboretum contains between 4,000 and 5,000 species; with all the disadvantages of an infertile soil and of London fog, it is probably without a rival in any other country.

Hooker carried on two periodicals illustrative of Kew work. The 'Botanical Magazine,' founded in 1787, had been edited by the elder Hooker from 1824. It consists of figures of new and interesting cultivated plants, and, after the association of the magazine with Kew, its collections chiefly supplied the materials. Hooker conducted it till 1904, writing mostly the descriptions himself. The "Icones Plantarum" had been started by the elder Hooker, and afterwards passed into Bentham's hands. It consists of figures of interesting and novel plants drawn from the Herbarium. Hooker edited the third series.

These were not the only burdens which a Kew position placed on Hooker's shoulders. The Government had given early encouragement (and some pecuniary aid) to the Hookers in the publication of Floras of British Possessions. The first of these was the elder Hooker's 'Flora Boreali-Americana' (1829–40). But this, like those included in the botany of the Antarctic voyage by the younger, were "on too expensive a scale to be generally useful." This led to Sir William Hooker suggesting in 1857 that the series should be continued in 8vo volumes in the English language, "scientific, yet intelligible to any man of ordinary education." The plan was adopted and sanctioned by the Duke of Newcastle in 1860. The scheme necessarily required the co-operation of many hands. Hooker co-operated actively. He assisted Thwaites in his 'Enumeratio Floræ Zeylaniæ' (1864), produced a 'Handbook of the New Zealand Flora' (1864–7), based on his own previous work, and edited a second edition of Harvey's 'Genera of South African Plants' (1868). In 1870 he published 'The Student's Flora of the British Islands' (3rd Edition, 1883) much on the same lines. This would have been the first British Flora to give the external geographical distribution if the 'Compendium' of H. C. Watson had not been published somewhat earlier in the same year. Hooker's original plan was large, but proved impracticable. Darwin, writing in 1868, said "It is a splendid scheme, and, if you only make a beginning on a Flora, which shall serve as an index to all papers on curious points in the life-history of plants, you will do an inestimable good service" (M.L., vol. 2, p. 373). He hoped at some time "to undertake such a task, in the form of a companion," but never accomplished it. In some degree, Lord Avebury's 'Notes on the Life-History of British Flowering Plants' (1905) fills the gap.

No very long time elapsed before the India Office also expressed the wish that the Indian Possessions of the Empire should be included in the scheme. In 1872 Hooker, acting under "instructions" from the Duke of Argyll, issued the first part of a 'Flora of British India.' Though on a more modest scale than the abortive attempt made by himself and Thomson in 1855, it was not completed, even with the assistance of many contributors, for a quarter of a century. But this will be referred to later. In 1877 he saw through the press Baker's 'Flora of Mauritius and the Seychelles.'

Something of personal history must be resumed. In 1860 Darwin wrote to Hooker, "Huxley is eager about a 'Natural History Review' which he and others are going to edit, and he has got so many first-rate assistants that I really believe he will make it a first-class production" (L.L., vol. 2, p. 328). It was an Irish venture transferred in a second series to England. Darwin told Huxley that, "I (Huxley) ought not to waste myself in other than original work" (H.L.L., vol. 1, p. 210). During its short but brilliant career—it died in 1865—Darwin was enthusiastic and even contributed a review. There is no doubt it was a very effective weapon in smiting what Huxley called the Amalekites (*loc. cit.*, vol. 1, p. 215). He regarded Hooker "as art and part of the 'Natural History Review,' though not ostensibly one of the gang" (*loc. cit.*, vol. 1, p. 237), and professed to be in terror of being "blown up" by him (*loc. cit.*, vol. 1, p. 246).

In the autumn of 1860 Hooker was invited by Captain (afterwards Admiral) Washington, the Hydrographer of the Navy, to accompany a scientific expedition to Palestine, to which Daniel Hanbury was also attached. This must have been thought to be not free from risk, even to so experienced a traveller. For Darwin called it astonishing (M.L., vol. 1, p. 166), implored him not to "get your throat cut," and thought he "must be a little insane" (L.L., vol. 2, p. 337). There seems to have been no adventure. One outcome was the well-known paper on "Cedars" in the 'Natural History Review.' They afforded a problem which Hooker had more than once discussed: a species with subordinate races in widely dissevered areas: Himalaya (Deodar), Syria (Lebanon), and Africa (Atlas). Nothing was accurately known previously of the grove on Lebanon, which he found to occupy an old moraine 4000 feet below the summit, which is no longer covered with perpetual snow. He thought that in a colder period the cedar would have descended to a lower level and been continuous through Afghanistan and Persia with the Himalayan race.

J. M. Cameron 1868

H. Dixon & Son. [illegible].

Returning to more personal events, Hooker gave in 1866 the lecture on "Insular Floras" at Nottingham, which has already been discussed. In the following year he made a brief excursion to Brittany with Huxley and Lord Avebury to examine pre-historic remains. Nothing refreshed him so much as a holiday of this sort with some scientific interest in view. In 1870 he went with Huxley to the Eifel; Tyndall would have been of the party, but was detained by his lectures. In 1873 he took Huxley, who had been seriously ill, to Auvergne. They visited the four volcanic areas explored by Scrope, and roughed it with all the hardihood of experienced travellers. Huxley was none the worse for sleeping on one occasion on two planks in a cupboard (H.L.L., vol. 1, p. 392). The friends thought that they had made a great discovery in the evidence of glacial action. Hooker published an account in 'Nature,' but found that he had been anticipated by Sir William Guise in 1870. In 1867, besides working out *Rosaceæ* for Martius' 'Flora Brasiliensis,' he edited a posthumous volume of his friend Boott's 'Illustrations of the Genus *Carex*.'

In 1868 Hooker was President of the British Association at Norwich. Darwin wrote that the address was received by the Press with "a chorus of praise," and that he himself thought it "most striking and excellent" (L.L., vol. 3, pp. 100–1). Hooker had, needlessly in the event, urged in extenuation of any shortcomings, want of leisure in the discharge of "duties as administrator of a large public department, entailing a ceaseless correspondence with the Government offices, and with botanical establishments all over the globe." He managed to touch on a wide range of important scientific questions. He lamented the failure of museum management to grasp its educational possibilities—and he might lament still. He reiterated the opinion, "shared by an overwhelming majority of British naturalists," that the National Collection of Natural History should be under the control of a scientific head, and this has come about. He dwelt with satisfaction on the fact that the ten years that had elapsed since the appearance of the 'Origin' found Natural Selection "an accepted doctrine with almost every philosophical naturalist." He thought Darwin's "new hypothesis of Pangenesis . . . may prove to contain the rationale of all the phenomena of reproduction and inheritance." The prevision was more correct than he really suspected to be probable; for as recently as 1909 Strasburger could say, "Charles Darwin's idea that invisible gemmules are the carriers of hereditary characters, and that they multiply by division, has been removed from the position of a provisional hypothesis to that of a well founded theory. It is supported by histology, and the results of experimental work in heredity, which are now assuming extraordinary prominence, are in close agreement with it" ('Darwin and Modern Science,' p. iii). Hooker thought that there were some who "will prefer embodying the idea in such a term as potentiality, a term which conveys no definite impression whatever, and they will like it none the less on this account." He was really quietly laughing at himself, for this is precisely what he and Huxley had propounded to Darwin, to whom it gave no comfort (L.L., vol. 3, p. 81). No doubt Pangenesis was in advance of its time, but, if we may measure the merit of a hypothesis by the depth of insight into the phenomena which it attempts to explain, Pangenesis is a greater intellectual feat than even Natural Selection itself.

The promulgation of Darwin's theory left Hooker's outlook on his own work substantially unaltered. But with Lyell the case was different. Though he did not realise it, we must agree with Huxley that, "consistent uniformitarianism postulates evolution as much in the organic as in the inorganic world" (H.L.L., vol. 1, p. 169). Darwin fully admitted his own personal debt, for in the dedication of the 'Journal' he says: "The chief part of whatever scientific merit this journal and the other works of the author may possess has been derived from studying the well-known and admirable 'Principles of Geology.'" Huxley emphasises this: "Darwin's greatest work is the outcome of the unflinching application to Biology of the leading idea and the method applied in the 'Principles'" ('Roy. Soc. Proc.,' vol. 44, p. viii). There can be few positions more tragic in life than that of the master who is confuted by his disciples from his own teaching. Yet this was Lyell's fate, and his conversion was slow and painful. In a passage which Darwin thought "felicitous and eloquent" (L.L., vol. 3, p. 101) Hooker did him justice. "I know no brighter example of heroism, of its kind, than this, of an author thus abandoning, late in life, a theory which he had for forty years regarded as one of the foundation stones of a work that had given him the highest possible position attainable amongst contemporary writers. Well may he be proud of a superstructure raised on the foundation of an insecure doctrine, when he finds that he can underpin it and substitute a new foundation; and, after all is finished, survey his edifice, not only more secure, but more harmonious in its proportions than it was before."

In 1869, at the instance of the Government, he attended the International Botanical Congress at St. Petersburg.

In 1871, Hooker, accompanied by his friends John Ball, the botanist, and George Maw, geologist, made (April to June) an important and successful expedition to Morocco, a country which, as he said then, "though close to Europe, is amongst the least known regions of the earth." He had been inspired with the idea of visiting it by Captain (afterwards Admiral) Washington, whom he accompanied to Syria in 1860, and who was "one of the very few Europeans who had reached the flanks of the Great Atlas Chain" (1829). The scientific problem which presented itself to Hooker was to "explore the great Atlas, to become acquainted with its vegetation, and to ascertain whether this supplies connecting links between that of the Mediterranean regions and the peculiar flora of the Canary Islands." Beyond the first two chapters, Hooker's other occupations prevented his writing the 'Journal of a Tour,' and it was completed by Ball (1878), but he supplied appendices in which he stated his botanical conclusions. The mountain flora of Morocco proved to be "a southern extension of the

European Temperate flora" (p. 445). The remarkable and unexpected fact was ascertained that the Alpine-Arctic flora had failed to cross the Western Mediterranean. The Great Atlas furnished "no gentian, no primrose or *Androsace*, no rhododendron, no anemone, no potentilla, and none but lowland forms of saxifrage and ranunculus" (p. 230). As to the Macaronesian flora, Hooker was finally "disposed to regard it as a very distinct subdivision of the Mediterranean province," in which "types once common to West Europe and North Africa" have been eliminated (p. 421).

The expedition was not free from difficulty and even danger, but escaped mishap. The Moroccan Government in assenting to an exploration of the Great Atlas stipulated that the ridge should not be crossed into the Sous Valley. The highest point reached was the Tagherot Pass (11,483 feet), which had never been reached before by any European. Incidentally they found that the practice of sacrificing animals as a propitiatory rite survived amongst the Berbers, and they were themselves on one occasion the object of it, in the hope of securing through their influence the release of men imprisoned by the Moors for non-payment of taxes. It is interesting to note that Hooker and Ball formed and expressed the conviction that "the one reasonable prospect of improvement in the condition of Morocco is to be sought in its passing under the control of a civilised state," and that it should be France (p. 351).

Kew had been re-organised under the Department of Woods and Forests with almost lavish generosity. In 1850 it passed to the control of the Office of Works, to which officially it was only a "Pleasure Ground," and which never felt much sympathy for its scientific character and functions. It is not wholly surprising, therefore, that in 1872 Hooker had what have been euphemistically described as "protracted differences" with Ayrton, the First Commissioner. Sir Algernon West, Gladstone's private secretary, claims to have "made peace between them," and thought Ayrton the "more reasonable man of the two" ('Recollections,' vol. 1, p. 14). That may have been the official view; but the differences were not adjusted without debates in both Houses of Parliament. The scientific world saw clearly that the real question involved was the breaking up of Kew as a scientific establishment, and gave Hooker its unflinching support. What was perhaps of more importance was that both Kew and Hooker were popular; public opinion declared itself on his side; Gladstone transferred Ayrton to another office, and the electorate dismissed him at the General Election in 1874 from political life.

In 1873 the Royal Society removed from the restricted accommodation it had received in Burlington House to the building it at present occupies in the Quadrangle. Hooker was elected President, Huxley being a joint secretary. It was to be expected that the association of two men of strong individuality and intense devotion to the interests of science should leave their mark on the work and history of the Society. Hooker's first aim was to bring it more into touch with the social life of the community. Tentative and informal evening receptions were held, and the success of these induced the Council in 1875 to establish the annual Ladies' Soirée.

In 1874 Hooker suffered a terrible blow in the sudden death of his wife (Henslow's eldest daughter) on November 13, without the smallest warning, and while he was away from home. This prevented him from presiding at the Anniversary Meeting, and what Huxley described as the "acephalous condition" of the Society cast a gloom over the following dinner. Mrs. Hooker was a singularly gifted woman, and what her companionship and help had been to her husband needs but a single illustration. When Darwin in 1854 received the 'Himalayan Journals,' he wrote, "I feel sure that the time will never come when you and Mrs. Hooker will not be proud to look back at the labour bestowed on these beautiful volumes" (L.L. vol. 1, p. 392).

Attention had been drawn to the limitation of admission to the Society by one of the Fellows at the Anniversary Meeting in 1874, and Hooker took "the earliest opportunity of submitting it to the deliberations of the Council." No change was made beyond taking away from peers and restricting to Privy Councillors the privilege of being proposed for election without being subject to selection. It was still thought that the association of scientific workers with "men of signal eminence in statesmanship, art, or letters was generally desirable." Hooker, like others, would probably have regretted that the foundation of the British Academy in great measure superseded this.

In 1876 the Challenger returned from the voyage round the world "originated" by the Royal Society and "crowned with complete success." In 1872 Hooker had drawn up suggestions for Moseley as to what might profitably be done in plant-collecting in the course of an expedition whose objects were oceanic research. Opportunity was well utilised, but it was thought advisable "to limit the Botanical Reports to a review of Insular floras, which came within the range of the expedition" ('Narrative,' vol. 1, p. 943). This was carried on at Kew with Hooker's advice in the 'Botany,' vol. 1, published in 1885. Hooker was Chairman of the Committee of Publication of the Reports, which sat from 1876 to 1895, and was responsible for the production of 50 illustrated volumes, the work of of 76 authors, at an expenditure defrayed from public funds of some £50,000. The result was probably unique in the history of expeditions.

In 1878 he laid down the office of President. He was "influenced by the consideration that, though wholly opposed to the view that the term of the Presidency of the Royal Society should be either short or definitely limited, this term should not be very long." But his main reason was the pressure of "official duties" and "scientific engagements." In his Anniversary Address, he made an announcement which gave him peculiar pleasure. The fact is singular that the Royal Society has never possessed more than a slender endowment. The amount of the fees contributed by the Fellows "occasionally prevented men of great merit from having their names brought forward as candidates." It was found that a sum of £10,000 would be "required for effecting any material reduction." This sum Hooker raised privately and almost single-handed in about a fortnight. He would tell the story of how, having got £1,000 from his old Glasgow friend, James Young, who had made a fortune from the Torbane Hill mineral, he communicated the fact and his object to the common friend of both, Sir Joseph Whitworth, and received the reply, "Not to be done by Jimmy," and a cheque for £2,000.

The address being in a sense valedictory, Hooker devoted it to a luminous summary of the striking achievements of science during the period of his Presidency. In dwelling more particularly on botanical advance, he could urge with truth "the excuse that there is, perhaps, no branch of research with the early progress of which this Society is more intimately connected," and he could point to the fact that vegetable histology began with Hooke and Grew, and cytology with Robert Brown.

This was in other ways a period of intense activity. In 1874 Hooker presided over the Department of Zoology and Botany of the British Association at Belfast. He chose, as the subject of his address, "The Carnivorous Habits of some of our Brother Organisms—Plants." This was a notable performance, as it was his one incursion into physiology. He reviewed all the known instances, and demonstrated from his own observations the occurrence of proteid digestion in pitcher-plants (*Nepenthes*), of which he had been the monographer. He concluded: "Though the processes of plant-nutrition are in general extremely different from those of animal-nutrition, and involve very simple compounds, yet . . . the protoplasm of plants is not absolutely prohibited from availing itself of food, such as that by which the protoplasm of animals is nourished, under which point of view these phenomena of carnivorous plants will find their place as one more link in the continuity of nature." The subject is of great theoretical interest, for it points to the fact that chlorophyllian assimilation from inorganic compounds, on which plants mainly depend, is a habit acquired since the primary stem of plant-life diverged from the primordial stock.

In 1877, at the close of the session of the Royal Society, Hooker obtained an extended leave of absence, to accept an invitation from Dr. Hayden, United States Geologist-in-charge of the United States Geological and Geographical Survey of the Territories, "to visit under his conduct the Rocky Mountains of Colorado and Utah, with the object of contributing to the record of the survey a report on the botany of those States." Prof. Asa Gray and Sir Richard Strachey were also members of the party. The Anniversary Address for 1877 was mainly devoted to a general account of the survey, with an indication of the particular problem which his own share in its work might throw light upon. This problem had been raised by Gray in 1858, when he first pointed out that the Asiatic affinities of the North American flora were to be found, not, as might be expected, on the Pacific, but on the Atlantic side of the Continent. Hooker's solution was first given in a lecture at the Royal Institution on April 12, 1878. The whole problem is too intricate to allow of more than the barest summary of its data. Comparing North America with Europe, it is obvious that in the one the mountain chains are longitudinal, in the other latitudinal. Florida is consequently exposed to northern blizzards, from which the European Riviera is immune. The Miocene flora had a circumpolar extension. It was driven south during the Glacial period. Practically exterminated in Europe, it partially survived in Eastern Asia, where the glaciation was less severe. Owing to its greater elevation the Glacial period was more intense and more prolonged in Western than in Eastern North America; in the former the Miocene flora was exterminated with a few exceptions such as *Sequoia* in California, and its place was ultimately taken by a flora of Mexican origin; in the latter it returned northward after a temporary retreat. The whole subject was discussed in detail by Hooker in a report published by the Department of the Interior of the United States Government in 1881.

He had secured the attachment of naturalists to the Transit of Venus Expeditions sent out in 1874, and the Rev. Mr. Eaton went to Kerguelen's Land, which had also been visited by the Challenger earlier in the same year, and where Hooker had himself collected in 1840. In 1879 he again discussed all the available material ('Phil. Trans.,' vol. 168). The Fuegian affinities of the flora were confirmed. "Winds almost throughout the year, blow from Fuegia to Kerguelen Island . . . . but appear quite insufficient to transport seeds over 4,000 miles." He still leaned, "as the forlorn hope of the botanical geographer," to his early belief in the former existence of more land between the two. On the other hand the remarkable fact which he had pointed out in 1875 ('Linn. Journ.,' vol. 14), that the scanty vegetation of Amsterdam Island and Tristan d'Acunha, 3,000 miles apart, "approximates to that of South Africa," may be due to transport by land-birds. He points out that "no trace of the mountain flora of South Africa has been found in any of the southern group of islands." And this is unfavourable to their having had any land connection with South Africa on the one hand, or a hypothetical northward extension of the Antarctic Continent on the other.

The 'Genera Plantarum' was completed in 1883, and with the eighties Hooker began a determined attack on the 'Flora of British India.' This was partly a labour of love, partly a service which he felt Kew had long owed to the Indian Empire. He had utilised the co-operation of other botanists, but gradually took the whole burden on to his own shoulders; vol. 3 appeared in 1882 and vol. 4 in 1885. About that time he showed some symptoms of failing health. He had never been content to live less than a full life, and this his medical advisers decided could not continue. He resigned the Directorship in 1885 and retired to a house he had built for himself at Sunningdale. While this relieved him of official routine and social distraction it enabled him to work at Kew several days a week in the room in the library formerly occupied by Bentham. Under more tranquil conditions health was happily completely restored. The 5th volume of the Flora was finished in 1890; the 6th in 1894, including the *Orchidaceæ*, to which he devoted two years' work, and the 7th in 1897.

In 1904, at the request of the India Office, he contributed to the

3rd Edition of the 'Imperial Gazetteer,' "A Sketch of the Flora of British India." While he found little occasion to modify what he had written forty years earlier, he was able to summarise the results of the Flora. The number of species of flowering plants actually described "approaches 17,000." In 1855 Hooker had concluded ('Intr. Ess.,' p. 91) that "*Orchideæ* appear to form a larger proportion of the flora of India that of any equally extensive country." This was verified, the number of species being some 1300, and with few exceptions these are peculiar to it. Previous conjecture had regarded *Leguminosæ* and *Gramineæ* as relatively deficient, but they proved to be the next largest elements in the flora. As regards the former this is not surprising, as in most large areas it is dominant. *Compositæ*, the largest of all orders of flowering plants, fill a subordinate place, as in most tropical countries. In 1905 Hooker furnished the descriptions to the plates of a "Century of Indian Orchids," in the 'Annals of the Royal Botanic Garden, Calcutta.'

In 1885, the last year of his directorship, he summarised, in the 30th Edition of the 'Official Guide to the Royal Botanic Gardens and Arboretum,' the work done under his superintendence since 1870, on replanting the Arboretum, and incidentally gave an admirable and critical account of the most important and interesting elements of the collection. In 1902 the number of hardy trees and shrubs enumerated in the 2nd Edition of the 'Hand-list of Trees and Shrubs (excluding Coniferæ)' amounted to about 4500. Hooker had contemplated the preparation of a catalogue of the Kew Pinetum. He drew up an introduction which, after revision by himself, was prefixed to the 'Hand-list of Coniferæ' in 1896.

In 1886 he worked out, in a paper contributed to the Linnean Society, the material of species of *Castilloa*, which had been obtained for the purpose of more accurately determining the source of Central American rubber.

In 1887 he revised the 5th Edition of Bentham's classical 'Handbook of the British Flora.' (He did the same with subsequent editions up to the 8th, in 1906.) Darwin tried its use and was "charmed with it" (L.L., vol. 2, p. 132). There can be no better book for those who begin the study of British plants, and no better introduction to the principles of their classification. Hooker's own 'Student's Flora' is better suited to those who are more advanced.

From 1886 to 1897, as has been seen, Hooker was continuously occupied with the three remaining volumes of the 'Flora of British India,' with such assistance as the Kew staff could give him in the preliminary arrangement of material. It was not to be expected that the period could be prolific in other ways. But he managed to devote an immense amount of time and labour, which it might have been wished he could have spared himself, to another task which in some degree he felt affection imposed upon him. Shortly before his death Darwin had expressed a wish, by aiding "in some way the scientific work carried on" at Kew, to show "his gratitude for the invaluable aid which for so many years he received from its Directors and its staff" (L.L., vol. 3, p. 352). The result of Darwin's munificence was the

'Index Kewensis.' Darwin had found the usefulness in his own work of Steudel's 'Nomenclator Botanicus,' an alphabetical list of specific names of plants, with their native countries. This, since its publication in 1841, had fallen completely out of date. Sir William Hooker, however, had commenced having an interleaved copy continuously posted up, and this had been carried on for the use of workers in the Herbarium. Darwin thought that it might be printed; but this was not found to be altogether practicable. Hooker having associated with himself John Ball and several members of the Kew staff, it was decided to base the work on the 'Genera Plantarum,' to which it would serve as a complement, and to give under each genus the published species, with the place of publication and their native countries. The work was entrusted to Mr. Daydon Jackson, who was occupied upon it for ten years. Printing was commenced in 1892 and completed in 1895. Hooker "most generously devoted an immense amount of time to the herculean and monotonous task of revision, and . . . . brought his vast personal knowledge to bear on the independent but by no means inconsiderable task of settling the geographical distribution" ('Kew Bull.,' 1893, p. 343). The result has been described justly as "a work with regard to which the feeling is one of incapacity to understand what its absence implied."

Hooker edited, in 1896, the journal kept by Sir Joseph Banks during Cook's first voyage round the world, which Admiral Wharton had described in his own preface to Cook's 'Journal' as "to the English nation the most momentous voyage of discovery that has ever taken place." Hooker's object was "to present him as the pioneer of those naturalist voyagers of later years, of whom Darwin is the great example." Dawson Turner, Hooker's maternal grandfather, who had been a friend of Banks, had undertaken to write his life, a task he never accomplished. Two of his daughters made a transcript of the 'Journal,' the original of which Hooker saw as a boy in 1833, when he was "fascinated" with it, and "never ceased to hope that it might one day be published." Banks' papers were for some time in the custody of the British Museum, but were ultimately sold by Lord Brabourne. Beyond the fact that Banks' Journal was purchased by a dealer for £7 2*s.* 6*d.*, its ultimate fate was unknown. As has since been ascertained, it has found an appropriate resting-place in the Mitchell Library at Sydney. The Turner transcript was retained at the British Museum, and Hooker was allowed to have it copied. In preparing it for the press he freely excised what was the mere record of trivial daily occurrence, and some matter too anthropological for general publication. The manuscript copy upon which he worked was presented by Mr. Reginald Hooker, who assisted his father, to the Kew Library.

Having finished the 'Flora of British India' as already stated, Hooker took up the completion of Trimen's 'Handbook to the Flora of Ceylon,' left unfinished by the author's death. This he completed in 1900, and in the 'Imperial Gazetteer of India' gave his final conclusions on the Indian flora in 1907.

His last literary effort was to fulfil a wish long entertained to write 'A Sketch of the Life and Labours' of his father, for whose memory he always cherished a deep affection ('Ann. of Bot.,' 1902). He dwells on "the solicitude with which he fostered my own aspirations to become a traveller and a botanist." It is rare in personal history for the lives of father and son to form a continuous whole. It was a relationship where, to use his own words, "one soweth, another reapeth."

Robert Brown was no less distinguished as a morphologist than as a systematist. Hooker, who took his place in English science, might have had equal fame in both had not his interest in classification and distribution been dominant. But his early palæontological work, in which the problems are purely morphological, proves that he had the root of the matter in him. No one, in fact, can accomplish anything fundamental in classification who has not the morphological instinct, and all through life the detailed study of plants with aberrant and peculiar structure always attracted him. His classical memoirs on *Balanophoreæ* (1856 and 1859), a group of parasitic and consequently highly reduced flowering plants, almost simulating fungi, raised a question as to the homology of the female flower, which he thought could only be solved by the study of its development, but as to which 25 years afterwards he was not disposed to alter his views. The knot is now cut by regarding reduction as having been carried to a point where all homology has disappeared. In 1857 he discussed the anomalous floral structure of *Siphonodon.* In 1859 he took up the study of *Nepenthaceæ*, a family which he monographed for De Candolle's 'Prodromus' in 1873. His theory of the pitcher (which was reproduced in French) as the expansion of an apical leaf-gland is, in different terms, that substantially accepted. In 1863 he produced his great paper on *Welwitschia* ('Linn. Trans.,' vol. 24) (reproduced in German and Portuguese), which alone would have made the fame of most botanists. Asa Gray thought that it was "the most wonderful discovery, in a botanical point of view," of the century, and that Hooker had "enjoyed (and improved) an opportunity unequalled by any botanist since that which placed *Rafflesia* in Mr. Brown's hands."

This extraordinary plant in its extravagant anomaly is unique amongst ligneous plants. Its stem instead of growing vertically expands horizontally like a huge fungus half buried in the sands of South Africa, on which it throws out its single pair of strap-shaped leaves. These grow continuously from the base, to die off at the apex; Hooker not unnaturally regarded them as permanent cotyledons. In 1880, when seedlings had been raised at Kew, Bower found that this was not the case, and that the actual cotyledons were extremely fugitive, while the permanent leaves were a second pair at right angles to them. This, though important and accepted by Hooker ('Gen. Pl.,' vol. 3, p. 1224), in no way diminished their anomalous character. It is noteworthy that in after years Hooker had occasion to describe species of a widely different South African genus, *Streptocarpus*, in which the single leaf is actually an enormously developed cotyledon.

That was a time of stress; Hooker wrote to Darwin, "I am plodding away at *Welwitschia* by night and 'Genera Plantarum' by day" (M.L., vol. 1, p. 467), Darwin thought he was over-working (*l.c.*, vol. 2, p. 284). The problem was to determine the affinity underlying such extreme adaptive disguise. Darwin said, "I see plainly that *Welwitschia* will be a case of Barnacles," *i.e.*, the *Cirripedia*, his own study (M.L., vol. 1, p. 213). As an archaic survival it "seems to be a vegetable *Ornithorhyncus*, and, indeed, more than that" (M.L., vol. 2, p. 281). Hooker placed it in *Gnetaceæ*, a perplexing group standing midway between Gymnosperms and Angiosperms, and there it remains. Later botanists have disputed as to the nature of the ovular integuments; but it may be doubted whether this involves more than verbal distinctions.

Hooker was now more and more absorbed by his larger undertakings, and could spare little time for researches not ancillary to them. Two may be mentioned. In 1875 he made a careful study of *Prosopanche*, of which a solitary South American species represents the strange African parasites, *Hydnora*. He contributed to the 'Annals of Botany' in 1887 a description of *Hydrothrix*, a new genus of *Pontederiaceæ*, which had escaped notice in the 'Genera,' founded on an amphibious Brazilian plant, exceptional amongst its allies in its single-stamened flowers.

Balsams (*Impatiens*) had always attracted Hooker, no doubt from the morphological interest of their floral structure, and the extreme difficulty of studying it in a dried state, which makes them the despair of herbarium students. He described the Indian species in 1860 and worked them over again for the 'Flora of British India' in 1875, and once more for the 'Records of the Botanical Survey of India,' from 1904–6. The genus continued to furnish the recreation and occupation of his remaining years. Hooker turned his attention to China, which, when opened up to collectors, proved extremely rich in new species of the genus. Foreign herbaria were only too glad to put their material at his disposal. The results of his studies, published from time to time, were the subject of no less than 14 papers. In one in French, contributed in 1908 to the 'Nouvelles Archives du Muséum,' he described the species in the Paris Herbarium. He was then able to say that, while in 1862 135 species were known, he was now able to recognise some 500. The difficulty of the task he had imposed upon himself may be judged from his remark: "Celui qui étudie ce genre sur le sec apprend vite qu'il n'y a pas de plus grand difficulté pour un systematicien que l'analyse, la coordination et la description des *Impatiens*." The process of soaking and laying out the flowers for examination was extremely tedious and required great skill; "chaque pli doit disparaitre; et cela souvent est une opération si longue qu'une heure et souvent deux ou trois suffisent à peine pour une fleur." Hooker still continued his work on *Impatiens*, till his forces finally failed him, and its last instalment, with dissections drawn, as in others, by his own hand, did not appear till shortly after his death. He was unable to frame a classification which entirely satisfied him, but he was able to establish the remarkable fact that the species are grouped in geographical areas which only rarely have any in common. It is obvious that we have in *Impatiens* a case of specific evolution, which deserves and awaits further and profound study.

Hooker was twice married: first, 1851, to Frances Harriet (died 1874), eldest daughter of Rev. John Stevens Henslow, by whom he left four sons and two surviving daughters; second, 1876, to Hyacinth, only daughter of Rev. W. S. Symonds, widow of Sir W. Jardine, seventh baronet (died 1874), by whom he left two sons.

Hooker was 5 feet 11 inches in height, and throughout life spare and wiry in figure. In 1859 Huxley wrote to him: "Don't let all the flesh be worried off your bones (there isn't much as it is)." There was a touch of the "quarter-deck" in his carriage. With a fresh complexion, there was in later life a general ruggedness in his aspect, and he was somewhat indifferent as to his personal appearance. Those who knew him in middle age think the published photograph by Mrs. Cameron most characteristic. There are portraits by Collier at the Royal Society and by Herkomer at the Linnean. Huxley wrote of this in 1859 (H.L.L., vol. 2, p. 232), "You were never quite so fat in the cheeks," and quizzed him about the fur coat; it certainly gave an aldermanic effect wholly foreign to him. The portrait has been reproduced. There is a bronze medallion modelled from life by Frank Bowcher.

Great powers of physical endurance carried him through his travels with no permanent injury to health. His temperament was nervous and high-strung, and he could not stand petty worries, especially those incidental to official life. A somewhat strict disciplinarian, he always retained the sense of official subordination, which he had no doubt learnt in his naval service. He was careful to note that he acted "under instructions," and would speculate as to what the "Board" would say in the smallest administrative details. His outlook in life was calm and philosophic; science and its progress was his absorbing and single-minded interest. He would spare no pains to advance it. The x Club kept him in touch with what was being done outside his own subject. Geographical research was with him a passion only second to botany. Had he not been a great botanist, he might have been an even greater geographer.

He had a great gift for securing and retaining friendship, which was helped by a keen sense of humour and an admirable gift in letter-writing, which he doubtless inherited from his maternal ancestors. He was a vivacious conversationalist, fond of paradox for the sake of stimulating discussion, but was little prone to draw on his past experiences.

He was nervous about public speaking, but when pushed could make an excellent speech, rising to some eloquence. That when his health was proposed as Copley Medallist was a notable performance, reviewing with a dignified modesty the "incidents that directed my own scientific life," and concluding that they "are fruitless, if there is not some inward motive power to compel us to exercise our faculties, and some inward heat, some fervour, to ripen the fruits of our labours." In his own case, he was content to have found it in the motto of Prince Henry of Portugal, the father of navigation, "talent de bien faire"—"the wish to do well."

His literary style in early life was laboured and sometimes obscure, but later became nervous and precise, and he was singularly happy, especially in technical matters, in seizing the felicitous and pregnant word. Though as a young man he had beguiled a tedious journey through the Sundarbans with Tennyson's "Princess," poetry had no appeal to him in after-life. But artistic tastes, inherited from both parents, were not atrophied. He was an accurate and more than ordinarily skilful draughtsman. He was an ardent collector of Wedgwood, and the severity of Flaxman's line and composition particularly appealed to him. He derived much pleasure at home and abroad from the older masters, and much admired the modern French school before its later developments. He continued to enjoy music of a classical type throughout.

A few words may be said to indulge a reasonable curiosity as to the physical conditions of a life in which so much was accomplished and which was so prolonged. He was very abstemious and smoked only moderately, but never at work. He could dispense with sleep to a remarkable extent. In his prime he would work till two, to wake at five and read in bed till seven, a habit he had contracted as a student. On the outward voyage the Erebus stopped at Madeira, and Hooker eagerly explored its flora. Sleeping out under a tree he contracted rheumatic fever. The Antarctic voyage must have tried him, followed by the labour of working out its results, for Darwin reminded him in 1860, "that you were bad enough before your Indian journey" (L.L., vol. 2, p. 203). The "troubled heart-action" about which Darwin wrote to him seriously the previous year (M.L., vol. 1, p. 98) was doubtless due to the rheumatic fever. Of this he had a more severe and happily final attack in 1865 when he was carried in blankets by four men to see from a window his father's body leave the house. In 1885 there was a return of heart trouble and marked deterioration of the arteries. His retirement from official life was followed by complete restoration to health, which was henceforth undisturbed except by some troublesome gouty ailments in his last years. His mental powers retained unabated vigour and activity to the end. The summer of 1911 enfeebled him. What seemed a temporary illness compelled him at the last to remain in bed. In the last week he asked that the account of the Royal Society dinner should be read to him. The day of his death excited no anxiety. He passed away in his sleep at midnight on December 10.

The Dean and Chapter of Westminster offered, with public approval, the last supreme honour of burial in the Abbey. It would have been fitting that his ashes should be placed near Darwin. But at his own expressed wish he was taken back to Kew, the scene of his labours, and there on December 15 he was laid to rest in the grave which contained the remains of his father and of his first wife.

Hooker's long life was, as it deserved to be, punctuated with honours. He received honorary degrees from Oxford, Cambridge, Dublin, Edinburgh, and

Glasgow. In 1869, after his Presidency of the British Association, he was created C.B.; in 1877, at the close of that of the Royal Society, K.C.S.I.; in 1897, on the completion of the 'Flora of British India,' he was advanced to G.C.S.I. in the Diamond Jubilee list of honours, his old friend Sir Richard Strachey receiving the same distinction. In 1907, on his ninetieth birthday, the Order of Merit was handed to him personally at his home on behalf of the King. As an old naval officer he felt himself debarred from accepting foreign decorations. The apparent exception was the Order of the Polar Star; Hooker explained his position to the Swedish Ambassador and, while unable to accept the honour, was allowed to retain the decoration (H.L.L., vol. 1, p. 361). He accepted the Prussian "Pour le Mérite," but only after the King's express approval; but that, as Huxley said, "is a purely literary and scientific affair."

From the Royal Society Hooker received a Royal Medal in 1854, the Copley in 1887, and the Darwin in 1892, the second occasion of its award. The Society of Arts gave him their Albert Medal in 1883; the Geographical their Founder's in 1884, and the Manchester Philosophical the Wilde Medal in 1898. From the Linnean Society he received the Linnean Medal in 1888, one specially struck "on the occasion of the completion of the 'Flora of British India'" in 1898, and in 1908 that struck on the jubilee of Darwin's memorable communication. In 1907 he was the sole recipient from the Royal Swedish Academy of the Medal to commemorate the bicentenary of the birth of Linnæus, and this, short of the Order of Merit, which came afterwards, he regarded as "the crowning honour of his long life."

He was one of the eight Associés Étrangers of the French Académie des Sciences and a member of other scientific societies throughout the world too numerous to mention.

A life which has been contemporary with three generations requires as many portraits to show the personality which was familiar to each in succession. The earliest of those reproduced shows Hooker in his 38th year, and is reduced from a chalk drawing by George Richmond, in the possession of C. P. Hooker, Esq., of Dollarward House, Cirencester. It is reminiscent of the "most engaging young man" whom Darwin had described to Lyell eleven years earlier (M.L., vol. 2, p. 120). In middle life it has been difficult to find anything satisfactory; he was too busy to think about portraiture. Perhaps the most characteristic is Mrs. Cameron's photograph, which has been reduced. Of this Darwin wrote (M.L., vol. 2, p. 376): "I have got your photograph over my chimney-piece and like it much; but you look down so sharp on me that I shall never be bold enough to wriggle myself out of any contradiction." Though only 13 years later than the Richmond portrait, it is eloquent of the stress of official labour. The last portrait is from a photograph taken in the last year of his life in his garden at Sunningdale.

W. T. T.-D.

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